ESTIMATION OF SALIVARY IMMUNOGLOBULINS AND ACUTE PHASE PROTEINS IN PATIENTS WITH POTENTIALLY MALIGNANT DISORDERS,

ORAL CANCER AND TREATED ORAL CANCER

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CERTIFICATE

This is to certify that this dissertation titled **"Estimation of Salivary Immunoglobulins and Acute Phase Proteins in Patients with Potentially Malignant Disorders, Oral Cancer and Treated Oral Cancer"** is a bonafide record of work done by **"Dr.S.ASWINI"** under my guidance during her post-graduate study period between 2008 – 2011.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY** in partial fulfillment for the Degree of **Master of Dental Surgery in Branch IX – Oral Medicine and Radiology.**

It has not been submitted (partial or full) for the award of any other Degree or Diploma.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	EXPANSION
1	A_1	Absorbance 1
2	A_2	Absorbance 2
3	APRPs	Acute Phase Reactant Proteins
4	CA-15-3	Carbohydrate Antigen
5	CEA	Carcino Embryonic Antigen
6	CONJ	Conjugate
7	CRP	C-Reactive Protein
8	CTRL	Controls
9	ELISA	Enzyme-Linked-Immuno-Sorbent-Assay
10	GCF	Gingival Crevicular Fluid
11	GTD	Gestational Trophoblastic Disease
12	HCG	Human Chorionic Gonadotropin
13	IL-6	Interleukin – 6
14	OD	Optical Density
15	OR	Odds Ratio
16	OSCC	Oral squamous cell carcinoma
17	OSMF	Oral Submucous Fibrosis
18	PCR	Polymerase Chain Reaction
19	PSA	Prostate Specific Antigen

20	R_1	Reagent 1
21	R_2	Reagent 2
22	S-IgA	Secretory Immunoglobulin A
23	S-IgG	Secretory Immunoglobulin G
24	ST	Smokeless Tobacco
25	STD	Standard
26	TMB	Tetramethylbenzidine.
27	TPA	Tissue Polypeptide Antigen
28	TSA	Tissue Polypeptide-Specific Antigen
29	WHO	World Health Organisation.
30	WHOSEA	Women's Health in South East Asia.

Oral cancer is the sixth most common cancer worldwide and continues to be the most prevalent cancer related to the consumption of tobacco, alcohol and other carcinogenic substances. Oral squamous cell carcinoma (OSCC) accounts for nearly 50% of all newly diagnosed cancers in India. The prognosis of this cancer remains relatively unchanged for the past 30 years, despite advances in diagnosis and management.[46]

The absence of definite early warning signs for most head and neck cancers suggests that sensitive and specific biomarkers are likely to be important in screening high-risk patients. Therefore, the dentist must consider all patients at risk and act accordingly in the history-taking and examination phases of the dental visit. By recognizing and establishing a diagnosis of oral cancer development in its early phase, the clinician can help the patient greatly increase his or her chances for a cure and a normal, full life. On the other hand, a much poorer outcome results when presentation and diagnosis are established at a later and more advanced stage. As clinicians, we can greatly influence disease outcome and quality of life when we confront oral mucosal alterations representing early squamous cell carcinoma in the patients.[18, 36]

Salivary analysis holds promise as a non-invasive approach to identify biomarkers for human oral cancer. "Saliva is a mirror of our blood," said Wong. The salivary glands are exocrine glands that produce protein profiles indicative of the individual's status at the moment of collection.

This potentially may be an advantage when seeking biomarkers for various diseases.[47]

As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected non-invasively by individuals with modest training. Furthermore, saliva may provide a cost-effective approach for the screening of large populations. Whole saliva (mixed saliva) is a mixture of oral fluids and includes secretions from both the major and minor salivary glands, in addition to several constituents of non-salivary origin, such as gingival crevicular fluid (GCF), expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, other cellular components, and food debris. Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use.[23]

Tumor markers that can be identified in saliva may be potentially useful for screening for malignant diseases. Salivary diagnosis may be part of a comprehensive diagnostic panel that will provide improved sensitivity and specificity in the detection of malignant diseases and will assist in monitoring the efficacy of treatment. Additional studies are certainly required to determine which salivary markers can be used for these

diagnostic purposes, and to determine their diagnostic value in comparison with other, more established, diagnostic tests. [23]

Salivary immunoglobulins play a role in host anti-tumor surveillance mechanisms. The predominant immunoglobulin in saliva is IgA. Approximately 90% of IgA in saliva is present as secretory IgA (S-IgA). S-IgA has properties such as inhibition of bacterial adherence, neutralisation of toxins, and prevention of absorption of antigens through mucosal surfaces. It should be noted that there are often very large differences and variations in the content of S-IgA. Secretary immunoglobulin G (S-IgG) is present in saliva in very low quantities. The physiological and immunochemical characteristics are similar to those of serum IgG. It is thought that S-lgG may act within the mucosal epithelium and lamina propria to neutralise antigens and promote their phagocytosis once they have gained entry.[74]

The Acute Phase Reactant Proteins (APRPs) are group of plasma proteins that alter their concentration in response to varied diseases. The levels of APRPs were shown to reflect events in tumour invasion and metastasis and were used in biochemical monitoring of cancer. Although extensive studies have been done in other cancers oral cancer has received less attention. These proteins are believed to play a role in regulation of the complex host response to malignancies.[44]

The salivary immunoglobulins and acute phase proteins levels may be altered in potentially malignant disorders and in oral carcinoma which may represent a local immunological attempt by the body to eliminate neoplastic cells.[49]

Although various studies have been done to emphasize the role of serum immunoglobulins and acute phase proteins in oral carcinoma, very few studies are done to demonstrate the changes in the concentration of immunoglobulins and acute phase proteins in saliva of patients with potentially malignant disorders and oral carcinoma.

This study deals with the salivary analysis to evaluate the immunological parameters (IgA, IgG, C-reactive protein and haptoglobin) in the saliva of patients with potentially malignant disorders, oral squamous cell carcinoma (OSCC) and patients treated for oral squamous cell carcinoma.

AIM:

To evaluate the prognostic value of salivary immunoglobulins and acute phase proteins in oral cancer.

OBJECTIVES:

1. To determine the levels of IgA, IgG, C-reactive protein and haptoglobin in the saliva of patients with potentially malignant disorders.
2. To determine the levels of IgA, IgG, C-reactive protein and haptoglobin in the saliva of patients with untreated oral squamous cell carcinoma.
3. To determine the levels of IgA, IgG, C-reactive protein and haptoglobin in the saliva of patients with treated oral squamous cell carcinoma.
4. To compare these levels with the control group.

The study is about the Evaluation of Salivary IgA, IgG, C- reactive protein and Haptoglobin in Potentially malignant disorders like Leukoplakia and Oral Submucous fibrosis, Oral cancer and Treated Oral cancer in comparison with healthy controls. The present literature review is about the various aspects of potentially malignant disorders - Leukoplakia and Oral Submucous Fibrosis, Oral cancer and their correlation with the salivary immunoglobulins and acute phase proteins levels.

ORAL PRE CANCER AND ORAL CANCER

The oldest description of human cancer was found in Egyptian papyri written between 3000-1500 BC. It referred to tumours of the breast. The oldest specimen of a human cancer was found in the remains of a female skull dating back to the Bronze Age (1900-1600 BC).The mummified skeletal remains of Peruvian Incas, dating back 2400 years ago, contained lesions suggestive of malignant melanoma. Evidence of cancer was found in fossilized bones and manuscripts of ancient Egypt. Cancer is not a disease of our modern industrialized age, as some may have believed at one time.[36]

One of the earliest human cancers found in the remains of mummies was a bone cancer suggestive of osteosarcoma. **Louis Leakey in 1932** found the oldest possible hominid malignant tumour from the remains of either a Homo erectus or an Australopithecus. This tumour was suggestive of a **Burkitt's** lymphoma (although that nomenclature was certainly not in

use then). Diseases that we know to be rare cancers today have had a long history.[36]

Hippocrates is credited with being the first to recognize the difference between benign and malignant tumours. His writings describe cancers of many body sites. The swollen blood vessels around the malignant tumours so reminded him of crab claws, he called the disease karkinos (the Greek name for crab). In English this term translates to carcinos or carcinoma.[36, 65]

The term tumour was originally applied to the swelling caused by inflammation. Neoplasm is a new growth that also may induce swelling, but by the long precedent the non – neoplastic usage had passed in limbo; thus the term is now equated with neoplasm.[65]

Willis in 1952 described neoplasm as an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissue and persists in the same excessive manner after cessation of the stimuli which evoked the change.[65]

DEMOGRAPHICAL REVIEW

Cancer is one of the major threats to public health in the developed world and increasingly in the developing world. In developed countries cancer is the second most common cause of death. According to the World Health Report 2004, cancer accounted for 7.1 million deaths in 2003 and it is estimated the overall number of new cases will rise by 50% in the next 20 years.[88]

There is a large geographical variation in oral cancer mortality. In the year 2000, oral cancer mortality rates were exceedingly high in some South Central Asian countries and Melanesia, where the rates were between 19 and 22 per 100 000 habitants. In Africa, oral cancer mortality rates have been as high as 14⁄100 000. In Europe, Slovakia and Hungary were the nations with the highest mortality rates (7 and 11 per 100 000). In America, oral cancer mortality rates were high in Bolivia, Paraguay, Uruguay and Brazil, with rates between 3 and 4 per 100 000 habitants, whereas countries with lower mortality were Salvador, Nicaragua and Ecuador, with mortality rates of 0.2, 0.5 and 0.7 per 100 000, respectively. [28,89]

In Mexico, oral cancer mortality rates were higher than pharyngeal cancer mortality. This was in agreement with other studies from several populations of the world (Canada, Colombia, New Zealand, Germany, the United Kingdom, Switzerland, Finland, Singapore, Japan, Israel and India), and in some areas of the USA. However, in other countries where the incidence of oral pre-cancer is high, like Northern France or Slovenia, pharyngeal cancer in men is equal or even more frequent than oral cancer.[27]

The prevalence of oral cancer is particularly high among men; oral cancer is the eighth most common cancer worldwide. Incidence rates for oral cancer vary in men from 1 to 10 cases per 100 000 population in many countries. In south-central Asia, cancer of the oral cavity ranks among the three most common types of cancer. In India, the age standardized incidence rate of oral cancer is 12.6 per 100 000 population.[87]

In the South Asian region over one-third of tobacco consumed is smokeless. Traditional forms like betel quid, tobacco with lime and tobacco tooth powder are, commonly used and the use of new products is increasing, not only among men but also among children, teenagers, women of reproductive age, medical and dental students. In India, where chewing tobacco is used with betel nuts and reverse smoking (placing the lit end in the mouth) is practiced, there is a striking incidence of oral cancer- these cases account for as many as 50% of all cancers.[88]

India has the highest rate of oral cancer in the world, caused by tobacco consumption. WHO research indicates a 500 percent increase in cancer by 2025, of which 220 will be due to tobacco use. According to Women's Health in South East Asia (WHOSEA), almost one-half of all cancer cases in men and one-quarter of all cancer cases in women in India are believed to be tobacco-related.[86]

Salonen et al in 1990 [68] reported on occurrence of oral mucosal lesions and the influence of tobacco habits in a randomly selected adult Swedish population. Nine hundred twenty (920, 95%) of the selected samples of 967 subjects comprising approximately 0.75% of the total adult population were examined; lesions were registered in 596 of the 920. The relationship between tobacco habits and mucosal lesions was analyzed and the time needed for treatment for the lesions was estimated. A positive correlation could be demonstrated between tobacco use and leukoplakia, frictional white lesion, coated tongue, hairy tongue and excessive melanin

pigmentation, while a negative correlation was observed for geographic tongue and aphthous ulcer.

Sankaranarayanan in 1990[69] found that oral cancer ranks number one among all cancers in male patients and number three among cancers in female patients. Causal association between oral cancer and the chewing of betel quid containing tobacco leaves or stem and other tobacco habits has been extensively established.

Chakrabarti et al 1991[14] in a Calcutta population compared the prevalence of oral carcinoma and dysplasia in smokeless tobacco users and non-users. A total of 3205 subjects were studied. Of the smokeless tobacco users, 1.96% had oral carcinoma compared with 0.36% of non-users. The prevalence of oral dysplasia in the user's group was 14.4% as compared with 6.85% in the group of non-users.

Warnakulasurya in 1991[82] reviewed the data on smoking and chewing habits that were prevalent in the rural population of SriLanka and provided an assessment of the risk from these habits for oral pre cancer. According to one study among 1133 villagers, 54% men, and 42% woman chewed betel quid out of which 46% of men and 63% of women included tobacco. Tobacco was chewed alone by 2.6%. A community based case control study conducted on oral precancerous lesion and condition in a screening camp included 359 patients (316 men and 43 women) aged over 20 years in whom the lesion was diagnosed and equal number of age and sex matched controls were included. The relative lowest risk of 5.3 among

men and 5 women were observed among chewers of betel quid without tobacco and were not significant. When the quid was chewed with tobacco the relative risk was 15 for men and 33 for women. Men who chewed betel quid with tobacco carried a higher risk than smokers (15 and 9.7 respectively). However a higher relative risk of 24.7 was seen among men who both smoked and chewed indicating a synergistic action.

Prabhu SR et al 1993[61] stated that oral cancer is currently the most frequent cause of cancer related death among Indian men, which is usually preceded by oral pre cancerous lesion like leukoplakia or condition like oral sub mucous fibrosis.

Gupta P.C. et al in 1998[34] conducted a house to house survey to investigate whether there was any increase in prevalence of oral submucous fibrosis (OSMF) and if so, could it be attributed to an increase in the use areca nut. A total of 11,262 men and 10,590 women aged 15 years and older were interviewed for their tobacco habits. Among 5018 men who reported the use of tobacco or areca nut, 164 were diagnosed as suffering from OSF with a prevalence of 3.2 %. Areca nut was used mostly in mawa; a mixture of tobacco, lime and areca nut and 10.9% of mawa users had OSF giving a relative risk of 75.6. The disease as well as areca nut use was concentrated in lower age group about 8.5% below 35 years and about 44% less than 25 years. The authors concluded that an increase in the prevalence of OSF, especially in the lower age groups was directly attributable to the use of areca nut products. They also pointed that this increased prevalence of areca

nut chewing and consequent increases in OSF was due to aggressive use of pan masala, which contains areca nut as a main constituent which may or may not contain tobacco.

Kayambe in 1999[43] performed a study in Congo and found low incidence of oral cancer compared to other countries. The relative frequency of oral cancer in Congo was about 2.1% and the palate was the most frequent site and squamous cell carcinoma was most common type of cancer. The most affected age group was between 50-59 years and women were more affected.

Crispian Scully et al in 2000[18] stated that the etiological factors of oral cancer (acting on a genetically susceptible individual) include tobacco use (75% of people with oral cancer smoke), betel use (Bidi leaf, and often tobacco, plus spices, slaked lime, and areca nut), alcohol consumption, a diet poor in fresh fruit and vegetables, infective agents (*Candida*, viruses), immune deficiency, and (in the case of lip carcinoma) exposure to sunlight.

Jeng et al in 2001[41] stated that betel quid chewing is widely prevalent oral habit in India, Taiwan, Papua New Guinea, South Asia, and South Africa. It has been estimated that 600 million people chew betel quid worldwide. An average of 15 to 20 quid had been chewed by the betel quid users daily. A casual link between betel quid chewing and oral diseases such as oral leukoplakia, oral submucous fibrosis and oral cancer had been strongly established.

Zain et al in 2001[92] stated about the role of tobacco smoking, chewing of tobacco, areca nut, and betel quid and drinking of alcohol are established cultural risk factors of oral pre-cancer and oral cancer worldwide. A geographic and regional variation in the prevalence of oral pre-cancer and oral cancer indicates that the socio cultural life style plays an important role in the etiology and pathogenesis of the disease.

Mehrota et al in 2003[51] stated that oral cancer was the commonest malignancy in Allahabad and the habit of chewing was particularly high among the oral cancer patients. The buccal mucosa was the most common site of oral cancer.

Ranganathan et al in 2004[63] performed a case control study to ascertain the various habits, including chewing and oral submucous fibrosis in a selected hospital based population in Chennai, India. A total of 185 consecutive patients with oral submucous fibrosis were matched with age and sex matched controls. There was a high preponderance of oral submucous fibrosis in males (9.9:1). Areca nut, pan masala and betel quid were associated with oral submucous fibrosis. Results showed that age, sex, smoking alone, alcohol use alone and smoking and alcohol alone were not associated with the development of oral submucous fibrosis. However the use of tobacco with alcohol with areca nut increases the risk.

Sinha in 2004[77] stated that oral use of smokeless tobacco is widely prevalent in the South East Asia Region; the different forms include chewing, sucking and applying tobacco preparations to the teeth and gums.

In Southeast Asia over 250 million people use Smokeless tobacco products; about 17% of total population in Southeast Asia uses oral tobacco; of which 95% belong to India (82%) and Bangladesh (13%) The global youth tobacco survey revealed high (10-20%) prevalence of smokeless tobacco use among young students (13-15 year)in Southeast Asia .Among disadvantaged youth group high (45%-71%) prevalence of tobacco use was reported in Southeast Asia . Tobacco is chewed in multiple forms in Southeast Asia, betel quid, leaf alone, leaf with lime and tobacco and areca nut preparation and tobacco water. Smokeless tobacco use varied from 7.2% to 59.4% in different states of India. In J & K, Goa, Himachal Pradesh, Haryana, Punjab, Kerala, Andhra Pradesh, Tamil Nadu, Delhi, Karnataka, Meghalaya, Rajasthan and West Bengal smoking prevailed over smokeless tobacco use while in Maharashtra, Uttar Pradesh, Sikkim, Madhya Pradesh, Assam, Orissa, Bihar, and Arunachal Pradesh smokeless tobacco use prevailed over smoking. In Gujarat, Manipur and Mizoram proportion of smoking and smokeless tobacco use, among males was almost equal (28.3 years. 29.4) while among female proportion was 5:1 (12.4% years. 2.5) in rural and urban areas respectively.

Warnukulasurya in 2004[83] reviewed different types of smokeless tobacco habits all around the world and its role in occurrence of oral cancer. There are two main types of smokeless tobacco (ST), chewing tobacco and snuff. It may be used alone or in combination with other substances. Chewing tobacco comes in various forms, loose leaf, plug or twist. Loose

leaf or dry powdered tobacco is often mixed with various ingredients according to the local custom. Snuff is commercially made in many different forms from fine cut or ground tobacco and can be dry or moist. Moist snuff is marketed as loose snuff in containers or as sachets (portion-bag). Many forms of ST are carcinogenic to humans and in animal studies. Cancer development at the site of placement and other oral mucosal lesions caused by these products has been described from several population groups.

Durazzo et al in 2005[21] performed a study in Brazil on 374 patients with oral squamous cell carcinoma. Their ages varied from 14 to 94 years (mean = 57.4 years), with 255 men (68.2%), and 295 out of 366 Caucasian (80.6%). A majority had tumours of the tongue and or floor of mouth (55.6%), while 20.3% had lip cancer. Squamous cell carcinoma was found in 90.3%, and glandular carcinoma in 4%, T4 tumours in 39.6%, T1 lesions in 15.2% of all patients. Nearly 62% had no regional metastases, and the relative incidence in young patients (40 years or younger) reached 8.6%, and concluded that in spite of the predominance of locally advanced tumours, a majority of patients had no neck metastases. The 31.8% incidence in females indicates an increasing incidence of oral cavity cancer among women when compared to previous periods at the same institution.

Neufeild in 2005[56] and his co workers conducted a survey in India between 1995-96 constituting 4, 71,143 subjects and stated that the prevalence of alcohol consumption was present in 4.5%, smoking of tobacco

was present in 16.2% and chewing of tobacco was present in 14% of the study subjects. The prevalence of these habits was found to be more common among men and among the rural population with no formal education.

Yi-Hsin-Yang et al in 2001[91] designed a population based survey to estimate the prevalence of oral submucous fibrosis (OSF), leukoplakia and various mucosal diseases and the usage of areca / betel quid chewing and to investigate the effects of areca / betel quid chewing on oral mucosal lesions in aboriginal community of Southern Taiwan. Three hundred and twelve people 20 years of age or older were included in the study. The areca / betel quid chewing habit was defined as chewing at least one quid a day for more than one month .Taiwan's chewing quid included the fresh unripe areca fruit, as an essential ingredient while tobacco is never added .The prevalence of chewing areca /betel quid was 69.5%with an average of 17.3 portions a day for an average of 24.4 years. More women (78.7%) than men (60.6%) chewed areca / betel quid .The prevalence of OSF was 17.6%, which was higher in women (19.5%) than in men (15.7%) while prevalence of leukoplakia was 24.4% with no difference between men and women .The prevalence of oral mucosal lesions increased as the years of chewing or daily consumption increased. It was found that the odds ratio for chewing areca / betel quid and having at least one of the above oral mucosal lesions was 8.21. Any additional smoking or drinking habits were not significant for having oral mucosal lesions. Gender was not a significant factor and age,

which was related to duration of chewing habit, had a significant impact on having lesions. The authors concluded that although the areca / betel quid in Taiwan does not contain any tobacco, a significant association was still identified between areca / betel quid chewing and oral mucosal lesions.

Saraswathi et al in 2006[70] stated that the habit of smoking, drinking and chewing tobacco products were common oral habits in India and these habits were positively related with development of oral lesion such as OSMF, leukoplakia and oral lichen planus which had potential for malignant transformation. In their study the prevalence of the habit of smoking was higher in the age of 20-31 years and 40-51 years. Prevalence of alcohol consumption was higher in the 21-30 years age group and prevalence of the chewing habit was highest in the 51-60 years age group.

Gunaseelan R et al in 2007[32] performed a community-based survey using qualitative methods. Out of 168 villages, 11 were randomly chosen. Fifteen in-depth interviews and five focus group discussions were conducted. Only those above 10 years of age and who used areca nut either in processed or un-processed form was included in the study. Among different forms of areca nut products, the use of *Hans,* which is a commercial flavoured product containing areca nut, tobacco and other ingredients is believed to be the most prevalent habit in all the age groups. Chewing areca nut is the initiating habit, leading to other habits such as smoking and consuming alcoholic beverages. Residents less than 30 years of age chew areca nut products for fun or because of peer pressure whereas

the 30 to 50 year olds get habituated due to reasons such as boredom and family problems. Although there is awareness regarding the ill-effects of tobacco use, there is not much awareness regarding areca nut. According to the residents, the community has not given enough thought regarding areca nut products and therefore no action has been taken to mitigate this high-risk behavior. It is the perception of the community that there is an increasing trend in the use of areca nut especially the commercial forms such as *Hans*, in rural TamilNadu.

Gloria et al in 2008[29] studied 1914 villagers in Colombia for the effect of reverse smoking the oral mucosa. Habit of smoking was present in 501 (26%) inhabitants' prevalence of reverse smoking was observed in 73 (15%) of the inhabitant. From the 46 persons clinically studied, 42 (91.3%) were female, with an average age of 59.3 years old (range 29 -85), 41 persons (89.1%) of the sample smokes tobacco, all of them combined their ways of smoking (conventional and reverse), the average number of tobaccos a day was 2.29 per day (range 1 – 7), and the average time that they have smoked was 30.83 years (range 1 – 65). In 20 (43.5%) cases both parents of the respondent were also in the habit of reverse smoking and the main motivations to acquire the habit were as follows: pleasure 21 (45.6%), not to smoke in the conventional way 8 (17.4%) and habit 7 (15.2%).

Mathew et al in 2008[50] studied total of 1190 subjects who visited the department of oral medicine and radiology for diagnosis of various oral complaints over a period of 3 months were interviewed and clinically

examined for oral mucosal lesions. The result showed the presence of one or more mucosal lesions in (41.2%) of the population. Fordyce's granules was observed most frequently (6.55%) followed by frictional keratosis (5.79%), fissured tongue (5.71%), leukoedema (3.78%), smoker's palate (2.77%), recurrent aphthae, oral submucous fibrosis (2.01%), oral malignancies (1.76%), leukoplakia (1.59%), median rhomboid glossitis (1.50%), candidiasis (1.3%), lichen planus (1.20%), varices (1.17%), traumatic ulcer and oral hairy leukoplakia (1.008%), denture stomatitis, geographic tongue, betel chewer's mucosa and irritational fibroma (0.84%), herpes labialis, angular cheilitis (0.58%), and mucocele (0.16%). Mucosal lesions like tobacco-related lesions (leukoplakia, smoker's palate, oral submucous fibrosis, and oral malignancies) were more prevalent among men than among women. Denture stomatitis, herpes labialis, and angular cheilitis occurred more frequently in the female population.

ORAL LEUKOPLAKIA

In ancient literature leukoplakia was first described by **Shushruta**, in 600 BC. who called it as Sanipataj Rog. Oral leukoplakia was first and fully described in the second half of the 19th century by the Hungarian dermatologist, **Schwimmer (1877)**. Over the ensuing decades, oral leukoplakia has been recognized and established as a definitively precancerous lesion, often serving as the harbinger for the development of oral cancer, its importance lying in the general morbidity and high mortality rates associated with invasive cancer.[40]

Various studies have established a definitive role of tobacco in development of leukoplakia. **Gupta et al in 1984[33]** stated that if there were no tobacco habit, there would be no leukoplakia.

Oral leukoplakia has been widely regarded as a pre cancerous lesion for many years. The term literally means white patch(Gk 'leucos' = white, 'plakia' = patch) and defined as a raised white patch of the oral mucosa measuring 5 mm or more, which cannot be scraped off and which cannot be attributed to any other diagnosable disease.[60]

Definition Of Leukoplakia:

In 1978 the World Health Organization collaborating centre for oral precancerous lesions defined leukoplakia as a white patch or plaque that cannot be characterized clinically or pathologically as any other disease.

Axell T et al in 1984[06] at the first international conference on oral leukoplakia at Malmo, Sweden defined leukoplakia as a white patch or plaque that cannot be characterized clinically or pathologically as any other disease and is not associated with any physical or chemical causative agent except use of tobacco.

Axell in 1994[07] defined leukoplakia as a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable disease.

Pindborg in 1997[60] defined leukoplakia as a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion.

Warnakulasurya in 2007[84] stated that leukoplakia should not be used to recognize white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer.

Prevalence/ Incidence:

Mehta FS et al in 1969[52] stated that males are predominantly affected but females are not immune. The male-to-female ratio was noted to vary enormously from district to district in India. In Gujarat and in Bihar, 95–98% of leukoplakias were seen in males, whereas in Kerala and in Andhra Pradesh, the proportions of lesions seen in males were 80% and 42%, respectively.

Axell T et al in 1987[06] found that white lesions were three times more common in males than in females. When proportioned according to probable etiology, the male to female ratio for tobacco-related leukoplakia was 6:1 while for idiopathic leukoplakia it was 5:2.

Jain et al in 1995[39] stated that incidence of oral leukoplakia was maximum in 5th and 6th decades of life. In a study conducted in Banaras, India 68 patients with oral leukoplakia with minimum age of 16 and maximum age of 67 are selected. Most common site for lesions was buccal mucosa (67.33% lesions) followed by labial mucosa (13.86% lesions) and tongue (11.88% lesions). Among three clinical types of leukoplakia;

homogenous type was present in 57.35% cases, verrucous type in 25% cases and speckled type in 17.65% cases. 26.48% patients were having oral submucous fibrosis along with leukoplakia lesions.

Etiology:

Silverman, in 1975[75] found that in Gujarat, 15% of 57518 mill workers did not habitually use tobacco or areca nut and only 2% of the 16 210 persons who had a mucosal lesion did not have a tobacco-related habit. The type of tobacco usage influences the distribution of the lesions: reverse cigar smoking causes lesions on the hard palate, chewing causes lesions at the site of quid placement and smoking of cheroots is associated with floor of mouth leukoplakia.

Pindborg et al in 1997[60] examined 1866 individuals and found that chewing betel quid is more predominant in older age group and among women. 13.1% of all individuals examined showed chewers mucosa, 12.4% leukoedema, 1.8% preleukoplakia and 1.1% leukoplakia. Positive correlation between leukoedema, preleukoplakia and leukoplakia and habit of smoking where found.

Wolfe et al in 1987[85] conducted a study among 226 Navajo Indians aged 14 - 19 years to assess the oral health effects of smokeless tobacco (ST). 64.2% of the subjects (75.4% of boys and 49% of girls) were users of ST and of these; over 95% used snuff alone or in combination with chewing tobacco. Leukoplakia was found in 40 subjects; 37 (25.5%) in ST users and 3 (3.7%) in non-users 29.6% of the boys who used ST had leukoplakia as

compared with 17% of the girls. All leukoplakia were found in the mandibular arch. The mean duration of use was longer in users with leukoplakia than in those without (3.5years Vs.2.9 years). 81.1% of the users with leukoplakia used ST one or more days per week, compared to 47.2% of those without leukoplakia. Thus the duration (in years) and frequency of ST use (days per week) were highly significant risk factors associated with leukoplakia. However the concomitant use of alcohol or cigarettes did not appear to increase the prevalence of these lesions. They concluded that in this study population, the odds of developing these lesions in users of ST were nearly nine times that of non users.

Robertson in 1990[66] conducted a study on oral mucosal lesions in smokeless tobacco users. The risk for oral mucosal lesions associated with the use of smokeless tobacco among 1109 professional baseball players was investigated. Leukoplakia was very strongly associated with the use of smokeless tobacco in this population of healthy young men. Of the 423 current smokeless tobacco users, 196 had leukoplakia compared to 7 of the 493 non-users. The amount of ST used (in hours per day that ST was held in the mouth), investigated frequency of ST use (hours since last use), type (snuff vs. ST), and brand of snuff used were significantly associated with risk for leukoplakia lesions among ST users.

ORAL SUBMUCOUS FIBROSIS

Oral submucous fibrosis is a chronic disorder characterized by fibrosis of the lining mucosa of the upper digestive tract involving the oral

cavity, oropharynx and frequently the upper third of the oesophagus. Except in early forms of the disease the clinical presentation is characteristic due to fibrosis of lamina propria.

Oral sub mucous fibrosis was first described by **Schwartz in 1952** under the term, atrophia idiopathia (tropica) mucosae oris. **Lal in 1953** described this condition as diffuse oral sub mucous fibrosis**. Su in 1954** described this condition as, idiopathic scleroderma of mouth. **Rao and Behl in 1962** described this condition as idiopathic palatal fibrosis and sclerosing stomatitis respectively.[61]

The pre cancerous nature of oral sub mucous fibrosis was first mentioned by **Paymaster in 1956** who observed the development of slowly growing squamous cell carcinoma in one third of his oral sub mucous fibrosis patient. Since then several studies performed on various population of different ethnic origin with habit chewing betel quid essentially with areca nut, with or without tobacco supported precancerous nature of oral sub mucous fibrosis.[61]

Definition of Oral Submucous Fibrosis:

It is an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx, preceded by and/ or associated with vesicle formation, always associated with juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat.[62]

Prevalence:

Gupta et al in 1980[33] calculated the incidence rate of OSF in Ernakulam, Kerala and found that it was 8 for men and 19 for women per 100,000.

Rajendran et al in 1986[62] did an epidemiological assessment of the prevalence of OSF among Indian villagers. He recorded a prevalence of 0.2% (n=10071) in Gujarat, 0.04% (n=10169) in Andra Pradesh, 0.07% (n=20388) in Bihar, 0.4% (n=1027) in Kerala and 0.03% (n=101761) in Maharashtra.

P.C.Gupta et al in 1998[34] reported the prevalence of Oral submucous fibrosis. A house to house survey was conducted in Bhavnagar district, Gujarat state. The use of areca nut containing products and tobacco was assessed through an interviewer administered questionnaire. The oral examination was done by dentist.

The diagnostic criterion for Oral sub mucous fibrosis was the presence of palpable fibrous bands. An increase in the prevalence of Oral submucous fibrosis in lower socio-economic status groups was observed.

Etiology:

Pindborg et al in 1984[59] performed a study on 100 subjects for their smoking and chewing habits and the condition of their oral mucosa. Out of 100 subjects 44 were male and 56 female. The dominating habits in the male were areca nut chewing and cigarette smoking and areca nut chewing and

water pipe smoking in female population with no additional habit. The predominant occurrence of oral submucous fibrosis was seen.

Rajendran.R in 1986[62] reviewed the etiology and pathogenesis of Oral submucous fibrosis. According to him oral sub mucous fibrosis, a pre cancerous condition of the oral cavity has been studied by a number of workers in the field. The available epidemiological data showed a clear cut geographical and ethnic pre dispositions, which suggested that certain customs or habits (chewing) prevalent among the population groups in South East Asia might be possible etiological factors. However none of these customs was shown to be casually linked. This led some workers to consider the importance of systemic pre dispositions, in addition to the effects of local factors on the oral mucosa. More research is needed to elucidate this problem.

Pindborg et al in 1995[59] reviewed the etiology of oral submucous fibrosis, a high risk pre cancerous condition, predominantly affecting Indians. Conception of chilly was hypothesized as an etiologic factor on the basis of ecological observations and a solitary animal experimental study. Subsequent epidemiologic studies that included case-series reports, large cross-sectional surveys, case control studies, cohort and intervention studies have identified arecanut as the major etiologic factor. The role of genetic susceptibility and that of autoimmunity are receiving attention currently. Influence of nutritional factors if any remains unclear.

Reichart and Philipsen in 1998[64] reviewed the literature on betel chewer's mucosa and reported the following findings. Betel chewer's mucosa was first described and defined in 1971. The prelevance of the betel chewer's mucosa varies between 0.2 % to 60% in different studies from south to South East Asia. Women are more affected than the men with buccal mucosa most commonly affected area along with the edentulous alveolar process, margin of tongue and the labial mucosa. It is often found with leukodema and oral ulceration. The etiology of the lesion is traumatic and chemical and it is most likely not precancerous. Based on retrospective studies the authors suggested that betel chewer's mucosa might be precursor of oral submucous fibrosis.

Shah N, Sharma PP in 1998[73] conducted a study to identify the role of chewing and smoking habit in the etiology of oral submucous fibrosis. In this study 236 cases of oral submucous fibrosis were compared with 221 control subjects matched for age, sex and socioeconomic conditions. It was found that chewing of areca nut, quid and pan masala was directly related to oral submucous fibrosis and not a single case was found without any chewing habit. The study showed that the pan masala chewers develop oral submucous fibrosis in half the time taken by areca nut betel quid chewers. It was also found that duration of chewing was not significantly correlated but the frequency of chewing was directly correlated to manifestation of oral submucous fibrosis.

Yang et al in 2001[90] found that although the areca/betel quid in Taiwan does not contain any tobacco, a significant association was still identified between areca/betel chewing and oral sub mucous fibrosis in ethnic Taiwanese aborigines. In a population based study conducted in southern Taiwan 320 subjects of 20 years of age or older were reviewed. The prevalence of oral submucous fibrosis and leukoplakia were 17.6% and 24.4% respectively.

Chen et al in 2002[17] found that HPV and betel quid chewing are the two most important factors associated with oral squamous cell carcinoma. In situ PCR ISH hybridization was performed on the tumour specimens from 29 patients with oral squamous cell carcinoma and 29 mucosal specimens from normal healthy individuals. Their betel quid chewing and cigarette smoking habits are reviewed.

Avon SL in 2004[05] described various oral mucosal lesions like oral submucous fibrosis in association with the use of quid.

YANG et al in 2005[90] investigated the risk of areca / betel quid chewing with or without cigarette smoking on Oral submucous Fibrosis (OSF) and other oral mucosal lesions. A stratified case – control study was done which included 102 patients with oral mucosal lesions or OSF confirmed pathologically in the case group. They were separately analyzed for men and women investigating their risks. Age and sex matched individuals with no mucosal or lesions formed the control group. Among the 102 patients, 76 (74.5%) are women &26 are men (25.5%). OSF was the

most prevalent disease (60.8%) followed by keratosis (39.2%) and epithelial hyperplasia (39.2%), squamous cell carcinoma in 9 cases and verrucous carcinoma in 2 cases. There were 73 of 102 patients (71.6%) with only area / betel quid chewing habit and without any cigarette smoking habit. OSF was the most prevalent in combination of area / betel quid chewing and smoking habits, hyperkeratosis in chewing areca/ betel quid without cigarette smoking group and squamous cell carcinoma in the quid only group with higher prevalence of acanthosis in patients who chewed both betel quid &stem quid. For OSF, people with both smoking and chewing habits had a statistically significant odds ratio (OR) 8.68 and for people with chewing habit only and without any smoking habit the OR was 4.51. For other oral mucosal lesions, people with mixed habits and chewing only had significant risks (OR = 8.37 & 3.95 respectively). For both OSF and other oral lesions, the OR of mixed habits and chewing only were both higher in women than in men. Also it was found that the amount of more than 10-29 and 30 and more counts of quids per day had significant odds ratio for both OSF and oral mucosal lesions. The areca / betel quid used in Taiwan does not contain any tobacco products and still a statistically significant association was found with oral mucosal lesions and OSF.

Ahmad et al in 2006[02] conducted an etiological and epidemiological study of oral submucous fibrosis in Patna, Bihar. Total 157 cases of OSMF and 135 control subjects were selected for study in the period of 2002-2004. It was observed that male: female ratio was 2.7: 1. The youngest case of

OSMF was 11 year old and the oldest one was 54 years of age. Maximum numbers of cases were belonging to 21-40 years of age and they were belonging to low or middle socioeconomic class. Most of the OSMF cases used heavy spices and chillies, whereas control mild had spices and chillies. Gutkha was the most commonly used by the OSMF cases. Only 3 per cent did not use any gutkha or other areca nut product where as 80 per cent control did not have any chewing habit. The OSMF cases used gutkha and other products 2-10 pouches per day and kept in the mouth for 2-10 minutes and they were using since 2-4 years. Most of the OSMF cases kept gutkha in the buccal vestibule or they chewed and swallowed it, only a small number of patients chewed and spitted it out. It was also observed that OSMF developed on one side of the buccal vestibule where they kept the chew and other side was normal.

TUMOR MARKERS

Chan and Schwartz in 2002[15] defined tumour marker as a substance which can be measured in blood or other biological fluids and can be used to differentiate a tumour from normal tissue or to determine the presence of a tumour in a patient. A tumour marker can be produced by the tumour itself or by the host in response to a tumour.

Measurement of tumour markers can be used to monitor cancer, predict the therapeutic response and prognosis of cancer, and in some certain situations even screen and diagnose for cancer.

The first identified cancer marker was monoclonal immunoglobulin light chain discovered by **Bence-Jones in 1846**. This **Bence- Jones** protein is still in clinical use in the diagnosis and prediction of the therapeutic response in cases of multiple myeloma.[29]

The first modern tumour marker used to detect cancer was human chorionic gonadotropin (HCG), the substance doctors look for in pregnancy tests. Women whose pregnancy has ended but whose uterus continues to be enlarged are tested for the presence of HCG. A high level of HCG in the blood may indicate the presence of a cancer of the placenta called gestational trophoblastic disease (GTD).[29]

The first success in developing a blood test for a common cancer was in 1965, when carcinoembryonic antigen (CEA) was found in the blood of some patients with colon cancer**.**[29]

Diamandis et al in 2002[19] stated that subsequently the concentrations of many hormones, enzymes, and other proteins in biological fluids have been shown to reflect the presence of cancer in patients. **Greg et al in 2003**[31] described the role of serum tumour in early detection of various types of malignancy.

An ideal tumour marker should be specific for a certain type of cancer and be sensitive enough to detect small tumours and thus permit early diagnosis. Unfortunately most tumour markers do not fulfill these criteria.

REVIEWS ON TUMOUR MARKERS:

Hilkens et al in 1984[35] stated that large number of tumour markers have been used or proposed as markers for breast cancer. CA 15-3 is a carbohydrate antigen expressed in several glandular structures including the mammary gland.

Björklund in 1990[08] stated that instead of the many epitopes recognized in the TPA assay, the tissue polypeptide-specific antigen (TPS) assay measures only the cell proliferation related epitope M3.

Stenman et al. in 1991[78] stated that specificity could be clearly improved by measuring the proportion of the free PSA to PSA α1-antichymotrypsin complex in addition to total PSA.

Sundström and Stigbrand in 1994[79] measured Proteolytic fragments of cytokeratins 8, 18 and 19 in the tissue polypeptide antigen (TPA) assay.

Molina et al in 1995[54] stated that Carcinoembryonic antigen (CEA) levels in serum have been shown to be related to tumour size and nodal involvement of breast cancer. Simultaneous use of CEA and CA 15-3 allows early diagnosis of metastases in 60-80% of breast cancer patients.

Eskelinen et al. 1994[24] **and Eskelinen et al. 1997**[25] stated that Serum TPA and TPS have been shown to have only limited value in the diagnosis of breast cancer.

Fleisher et al. in 2002[26] stated that the Prostate-Specific Antigen (PSA) is the most important marker for the evaluation and even screening of prostate cancer. It is very sensitive but most of the patients with increased PSA values have benign prostatic hyperplasia.

Fleisher et al. 2002[26] stated that serum CEA in colorectal cancer can be used to monitor the response to therapy and to document the progressive course of the disease. One of the most widely used tumor markers for gynecological cancers is CA 125 which is used for estimating the prognosis and monitoring of ovarian cancer and monitoring patients with endometrial cancer. However, serum CA 125 levels may be elevated also in benign gynecologic conditions and during the first trimester in normal pregnancy.

Eliaz Kaufman et al[23] stated that tumour markers that can be identified in saliva may be potentially useful for screening for malignant diseases. Salivary diagnosis may be part of a comprehensive diagnostic panel that will provide improved sensitivity and specificity in the detection of malignant diseases and will assist in monitoring the efficacy of treatment.

Carmen Llena Puy et al in the year **2006**[13] found that in some malignant diseases, markers can be detected in the saliva, such as the presence of protein p53 antibodies in patients with oral squamous cell carcinoma, or high levels of defensin-1 positively correlated with the serum levels. The presence of the c-erbB-2 tumour marker in the saliva and blood serum of breast cancer patients and its absence in healthy women is a promising tool for the early detection of this disease. In ovarian cancer too,

the CA 125 marker can be detected in the saliva with greater specificity and less sensitivity than in serum.

Mahanz Saheb Jamee et al in the year 2007[48] found that the concentration of salivary IL-6 in oral squamous cell carcinoma patients was higher than control group.

Ahmadi Motemayal et al in 2010[02] found elevated levels of salivary defencine – 1 to be indicative of the presence of oral squamous cell carcinoma.

REVIEWS ON SALIVARY IMMUNOGLOBULINS (IgA & IgG) IN POTENTIALLY MALIGNANT DISORDERS (LEUKOPLAKIA / OSMF)

Abrol in 1975[01] reported a rise in the salivary IgG levels in patients with oral submucous fibrosis.

Phatak AG and Gosavi DK in 1975 and Phatak AG in 1979[58] reported a rise in the serum and salivary IgG levels in patients with oral submucous fibrosis.

Thomas Loning et al in 1979[81] found that the incidence of immunoglobulins (IgA and IgG) was twice as high in those cases of leukoplakia where dysplasia was present.

Rajendran et al in 1986[62] found that there was no change in any of the immunoglobulin fractions in oral leukoplakia whereas a significant rise in IgA levels was observed in oral submucous fibrosis.

Gupta Dinesh Chandra et al in 1992[33] reviewed on the etiological factors of oral submucous fibrosis and found that in addition to iron and vitamin deficiency there was a genetic predisposition and an elevation of the IgG levels in patients with oral submucous fibrosis.

Krasteva.A et al in 2008[46] found that salivary IgA and IgG were significantly increased in patients with precancerous lesions.

REVIEWS ON SALIVARY IMMUNOGLOBULINS (IgA & IgG) IN ORAL SQUAMOUS CELL CARCINOMA

Mark.A et al in 1973[49] did a study in which the saliva of 260 patients were analyzed for immunoglobulin content in which 35 patients were affected by primary oral cancer. He found that the highest IgA titers were seen in patients with oral and pulmonary cancers.

Thomas Loning et al in 1979[81] found that the concentration of IgA and IgG decreased significantly with tumor dedifferentiation.

Rajendran et al in 1986[62] found a significant rise in IgA levels in oral cancer patients but found no alterations in IgG levels.

Rajendran and Vijayakumar et al in 1986[62] reported a rise in serum IgA in patients with oral squamous cell carcinoma and the levels were reported to be rising with the progression of the disease. These results reflect changes in the cell mediated immunity.

Hu – De – En et al in 1987[38] found that there was a tendency for higher levels of immunoglobulins to be associated with more advanced stage of carcinoma.

Kashmoola et al in 2001[42] and **Al-Rawi et al in 2005**[03] had showed that patients with oral squamous cell carcinoma had markedly increased salivary total protein concentration which may be due to increasing salivary immunoglobulins.

Robino Muchado de Souza et al in 2003[67] found that salivary IgA levels were reduced in cancer patients and were related to malnutrition, stress and tobacco. He found that the mean salivary IgA level was 17.0 ± 10.4mg/dL in controls and 13.7± 3.9 mg/dL in oral cancer patients.

Ashley M. Brown et al in 1975[04] stated that the IgA content of whole saliva of cancer patients was significantly elevated above that of controls. The results indicate that the elevated levels were attributable to leakage of serum factors through the damaged epithelium.

Shpitzer.T et al in 2007[74] utilized comprehensive salivary analysis to evaluate biochemical and immunological parameters in the saliva of oral squamous cell carcinoma patients. He found that the concentration of salivary IgG was higher (125%) in oral squamous cell carcinoma patients while the concentration of secretory IgA was lower (45%).

Krasteva.A et al in 2008[46] found that the salivary levels of IgA and IgG in patients with oral carcinoma were significantly increased. He stated that these findings reflect the local inflammation, accompanying the neoplastic process in the oral cavity. It could also be considered as being the local defense mechanism against tumour development.

REVIEWS ON SALIVARY IMMUNOGLOBULINS (IgA & IgG) IN TREATED ORAL SQUAMOUS CELL CARCINOMA

Einhorn et al in 1972[22] observed elevated levels of IgG at the time of completion of radiotherapy and higher levels 3 months after radiotherapy. Elevated levels of IgG demonstrated an immune response which is helpful in antibody-dependant cytotoxic cell killing of tumour cells. This may represent enhanced immunization by antigens released during radiation induced tumour breakdown.

Thomas Loning et al in 1979[81] found that the immunoglobulins (IgA and IgG) levels decreased after radiation therapy.

Brown et al in 1981[11] found that following radiotherapy changes in specific agglutination titers of oral isolates reflected changes in saliva IgA. He found 13 patients with increased salivary IgA and 23 patients with decreased salivary IgA when he assessed 36 patients over a 30 month post-irradiation period.

Hu De-En et al in 1987[38] reported that the levels of IgG decreased markedly after radiation therapy.

Jankovic.L et al in 1995[40] in his study on 40 patients with neoplastic disorders who have been treated has found that the mean IgG/IgA salivary ratio was 1.27 (normally below 1.0) due to an increased salivary concentration of IgG (mean 0.095 g/l), but also due to a decreased IgA concentration (mean 0.075 g/l); the IgG/IgA ratio in saliva was higher in patients with objective changes of the oral mucosa (1.53).

Meurman JH et al in 1997[53] found that the total salivary IgA decreased during cancer therapy which returned to the baseline level after termination of the treatment.

Krasteva.A et al in 2008[46] found that the salivary levels of IgG and IgA remain significantly higher ($p<0.05$) in treated cancer patients. These findings reflect the local inflammation, accompanying the neoplastic process in oral cavity.

REVIEWS ON ACUTE PHASE PROTEINS IN POTENTIALLY MALIGNANT DISORDERS, ORAL SQUAMOUS CELL CARCINOMA AND TREATED ORAL CARCINOMA

Gallo.O et al in 1994[30] conducted a study in 18 patients with oral squamous cell carcinoma and found an increase in IL-6 which in turn induces the synthesis of CRP in head and neck carcinoma and plays a role in the regulation of the complex host response to malignancies. Significant ($P < 0.0001$) relationships were found between IL-6 and CRP ($r = 0.69$).

Dritan Turhani et al in 2005[20] has demonstrated an increase in the levels of CRP in oral squamous cell carcinoma patients.

Krasteva.A et al in 2008[46] found that the salivary levels of haptoglobin and CRP were significantly higher in untreated oral squamous cell carcinoma. The high CRP levels are associated with tumour progression and poor survival. Increased levels of salivary haptoglobin could be related to direct transudation from the blood. He also stated that the CRP levels

remained higher in treated oral cancer patients and there was a significant decrease in the salivary haptoglobin in treated oral cancer patients.

Kelly Powell in 2009[44] analysed 10,408 patients in which 1624 patients had oral carcinoma. He found that patients with high baseline CRP (more than 3mg/L) had a 30 % greater risk of developing any cancer compared with those whose CRP was less than 1mg/L.

Sunil.D.Khandavilli et al in 2009[80] did a study was designed to establish if elevated preoperative levels of CRP could predict the prognosis of patients treated with primary surgery for oral squamous cell carcinoma (SCC). Sixty patients with oral SCC who were treated by primary surgery and microvascular free flap reconstruction, were included in the study. The relation between preoperative levels of CRP, clinicopathological features and patient prognosis was determined.

This study showed using bivariate analysis ($p = 0.003$) and multivariate analysis ($p < 0.001$) that a raised preoperative CRP was associated with worse overall survival. Tumour size and stage when combined with CRP levels increases the predictive power of this indicator.

Study topic: "Estimation of salivary immunoglobulins and acute phase proteins in patients with Potentially malignant disorders, Oral Cancer and Treated oral cancer"

Study design: The present study is a Randomized Control Study.

Study duration: This study was conducted between April 2009 to May 2010 in the department of Oral Medicine and Radiology of Ragas Dental College and Hospital, Dr. Rai Memorial Medical and Cancer Centre, Chennai.

Study population:

A total number of 80 patients were involved in the study.

Obtaining approval from the authorities:

Permission from the ethical committee of **Ragas Dental College and Hospital**, Chennai was obtained before starting the study.

Due consent to participate in the study was obtained from the Subjects in letter format both in Tamil and English.

MATERIALS

Examination of the patient

- Conventional Dental chair with halogen lamp
- A pair of sterile gloves and disposable mouth mask
- Stainless steel Kidney trays
- Plain mouth mirror, straight probe, tweezer

- Sterile gauze pieces and cotton
- Glass tumbler with water
- 0.2% chlorhexidine gluconate
- Sterilizer, cheatel forceps.
- Sterile plastic containers for collection of saliva.

METHODOLOGY:

Study Group:

The study group consists of a total number of 80 patients. Out of the 80 patients, 20 patients were suffering from Potentially malignant disorders like Leukoplakia and Oral submucous fibrosis, 20 patients were suffering from Oral cancer, 20 patients were treated for oral cancer and 20 were normal controls.

Group I Potentially malignant disorder (Leukoplakia & Oral submucous fibrosis):

This study group comprised of 20 patients visited the Department of Oral Medicine and Radiology. Among these patients 18 were males and 02 females with the age range from 21 – 75 years (mean 40.3 years) having Leukoplakia and Oral submucous fibrosis, diagnosed on the basis of clinical criteria.

Clinical selection criteria:

Leukoplakia: [60]

Leukoplakia is defined as a grayish white lesion of the oral mucosa that cannot be characterized clinically as any other definable lesion. It

appears as a white, elevated patch with a wrinkled surface. It can be homogeneous or non-homogeneous and can be as verrucous, papillary or nodular forms. On palpation the mucosa shows loss of suppleness, leathery with fissures called as cracked mud appearance and is rough in consistency.

Oral Sub Mucous Fibrosis: [34]

The patients were graded into one of the following 6 grades:

Grade I – Only blanching of the buccal mucosa without symptoms.

Grade II – Burning sensation, dryness of mouth, vesicles or ulcers in the mouth without tongue involvement.

Grade III – In addition to grade II there is restriction of mouth opening.

Grade IV – In addition to grade III palpable bands all over the mouth without tongue involvement.

Grade V – Grade IV plus tongue involvement.

Grade VI – Oral submucous Fibrosis with histologically proven oral cancer.

Group II (Oral Cancer):

This study group consists of 20 patients suffering from oral cancer diagnosed clinically. These patients were selected from the Department of Oral Medicine and Radiology, Dr.Rai Memorial Medical and Cancer center and Cancer shelter. Among these patients 15 were males and 05 females with the age range from 33 – 68 years (mean 48.45 years).

Clinical Selection Criteria:

Presence of a non – healing ulceroproliferative growth with pain, tenderness, limitation / loss of function, bleeding and indurated margins. Presence of regional lymphadenopathy.

Group III (Treated Oral Cancer):

This study group consists of 20 patients who have been treated for oral squamous cell carcinoma. These patients were selected from Dr.Rai Memorial Medical and Cancer center. Among these patients 14 were males and 06 were females with the age range from 24 - 69 years (mean 44.25 years).

Clinical Selection Criteria:

- Patients who have been treated for oral carcinoma.
- Patients who clinically shows no signs of carcinoma.

Group IV (Controls):

The control group comprises of 20 normal individuals who visited the outpatient department of Oral Medicine and Radiology. Among them, 15 were males and 05 females with the age range from 19 – 68 years (mean 40.5 years). Thus the control groups were matched with age and sex of the study group.

Clinical Selection Criteria:

- Patients who donot have any mucosal lesions.

- Individuals who are apparently healthy with no history of Diabetes, Hypertension and any known diseases.

Informed consent:

Informed consent was taken from all subjects before including them in the study.

Exclusion criteria:

Participants with infectious diseases during one month before saliva sampling, active dental abcesses, and collagen vascular diseases were excluded from the study.

Examination of the subjects:

The experimental subjects were made to sit comfortably on a dental chair. Sterile hand gloves were used during examination of the patients. Patients were examined under halogen lamp in the dental chair under aseptic conditions and relevant demographic data were collected. Clinical diagnosis was made and patients who showed characteristic features of Leukoplakia, Oral submucous fibrosis and Oral Cancer were prepared for sample collection.

Saliva sample collection:

The subjects were required to abstain from eating, drinking, smoking or using oral hygiene products for at least 1 hour before saliva collection. The patients were asked to rinse their mouth with water and were made to sit comfortably in a dental chair. Saliva was collected during a 15-minutes interval by spitting method. This was pooled saliva and represented the

output from all the salivary glands. 2-3mL of saliva was collected in sterile containers.

All samples were kept in ice after collection and then centrifuged at 3000 rpm for 10 min to remove particulate materials and the supernatant was used for estimation of the immunoglobulins and acute phase proteins.

QUANTITATIVE DETERMINATION OF SALIVARY IMMUNOGLOBULIN A BY ELISA METHOD. [37]

Principle:

Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) was used for the quantitative determination of **secretory IgA** in saliva. In a first incubation step, the sIgA in the samples is bound to polyclonal antibodies (rabbit anti human IgA), which are immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a Peroxidase-labeled conjugate (mouse anti-sIgA) is added which recognizes specifically the bound secretory IgA. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of secretory IgA. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated, using the results

obtained from the calibrators. Secretory IgA in the patient samples is determined directly from this curve.

Reagents:

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate 10x	1 x 100 ml
CONJ	Conjugate (mouse anti-sIgA, Peroxidase-labeled)	1 x 200 µl
STD	Calibrators, lyophilized (0; 22.2; 66.6; 200; 600 ng/ml)	2 x 5 vials
CTRL	Control, lyophilized	2 x 1 vial
SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	1 x 15 ml
STOP	ELISA stop solution, ready-to-use	1 x 15 ml

Saliva collection and preparation:

- No food or liquid should be consumed 30 min before sample collection.
- The samples were centrifuged at 3000 rpm for 10 min. Sample supernatant was stored at -20°C.
- For analysis, the supernatant was diluted 1:2000 in ELISA wash buffer, e.g.10 µl supernatant + 990 µl wash buffer; diluted the obtained solution again: 50 µl diluted supernatant + 950 µl wash buffer. 100 µl of the final dilution per well was used.

Procedure:

The precoated PLATE (microtiter plate) 5 x was washed with 250 µl ELISA wash buffer. The tests were carried out in duplicate.

1. 100 µl STD (standards), CTRL (control) and patient samples were added.
2. Incubated for 1 hour, shaking on a horizontal mixer, at room temperature.
3. Aspirated and washed the wells 5 x with 250 µl ELISA wash buffer.
4. 100 µl CONJ (conjugate; POD antibody) was added.
5. Incubated for 1 hour, shaking on a horizontal mixer, at room temperature.
6. Decanted the content of the plate and the wells 5 x were washed with 250 µl of wash buffer.
7. 100 µl SUB (TMB substrate) was added.
8. Incubated for 10-20 minutes at room temperature.
9. 50 µl STOP (ELISA stop solution) was added and mixed shortly.
10. Absorption was determined with an ELISA reader at 450 nm against 620 nm as reference.

For the calculation of the saliva values, the results from the microplate reader was multiplied by 2.000.

Range:

Children (n=37) 18 - 237µg/ml (mean 128 µg/ml)

Age >16 years (n=33) 102 - 471 µg/ml

QUANTITATIVE DETERMINATION OF SALIVARY IMMUNOGLOBULIN G BY IMMUNOTURBIDOMETRIC METHOD. [37]

Principle:

Anti-human IgG antibodies when mixed with samples containing IgG form insoluble complexes. These complexes cause an absorbance change, dependent upon the IgG concentration of the patients sample, that can be quantified by comparison from a calibrator of known IgG concentration.

Reagents:

Diluent (R1) - Tris buffer 20mmol/L, PEG 8000, pH 8.3.
Sodium azide 0.95g/L.

Antibody (R2) - Goat serum, anti-human IgG, pH 7.5.
Sodium azide 0.95g/L.

Calibration curve:

The following PROT CAL calibrator dilutions in NaCl 9g/L as diluent was prepared. The concentration of the IgG calibrator was multiplied by the corresponding factor stated in the table below to obtain the IgG concentration of each dilution.

Calibrator Dilution	1	2	3	4	5	6
Calibrator (µL)	-	10	25	50	75	100
NaCl 9g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

Equipment:

- Thermostatic bath at 37ºC.
- Spectrophotometer, thermostatable at 37ºC with a 600nm filter (580-620nm).

Procedure:

1. The reagents and the photometer (cuvette holder) were brought to 37ºC.
2. Assay conditions:

   Wavelength : 600 nm

   Temperature : 37 ºC

   Cuvette light path : 1cm
3. The instrument was adjusted to zero with distilled water.
4. Pipetted into a cuvette:

   Reagent R1 (µL) - 800 µL

   Sample or Calibrator (µL) - 10 µL
5. The reagents were mixed and the absorbance (A_1) was read after the sample addition.

6. Immediately, pipetted into the cuvette:

Reagent R2 (μL)	-	200

7. The reagents were mixed and the absorbance (A_2) of calibrators and sample were read exactly 2 minutes after the R2 addition.

Calculations:

The absorbance difference (A_2-A_1) of each point of the calibration curve was calculated and the values obtained were plotted against the IgG concentration of each calibrator dilution. IgG concentration in the sample was calculated by interpolation of its (A_2-A_1) in the calibration curve.

Reference value:

700 – 1600mg/dL.

Upto 3000mg/dL.

Detection limit:

Values less than 10.3mg/dL give non-reproducible results.

QUANTITATIVE DETERMINATION OF SALIVARY C-REACTIVE PROTEIN BY IMMUNOTURBIDOMETRIC METHOD [37]

Principle:

Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

Clinical Significance

CRP is an acute-phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial and virus infections, inflammation and malignant neoplasia. During tissue necrosis and inflammation resulting from microbial infections, the CRP concentration can rise up to 300 mg/L in 12-24 hours

Reagents:

Diluent – ultra (R1)	Tris buffer 20mmol/L, pH 8.2. Sodium azide 0.95g/L
Latex – ultra (R2)	Latex particles coated with goat IgG anti human CRP, pH 7.3, Sodium azide 0.95g/L
U-CRP CAL	Calibrator, C-Reactive protein concentration is stated on the vial label.

Preparation:

- Working reagent - The latex vial was gently shaken. 1mL latex reagent + 14mL diluent.
- CRP Calibrator - Reconstituted with 2.0mL of distilled water. Mixed gently and brought to room temperature before use.

Calibration Curve:

Prepared the following CRP Calibrator dilutions in NaCl 9 g/L as diluent. Multiplied the concentration of the CRP calibrator by the corresponding factor stated in table below to obtain the CRP concentration of each dilution.

Calibrator Dilution	1	2	3	4	5	6
Calibrator (µL)	-	10	25	50	75	100
NaCl 9g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

Equipment:

- Thermostatic bath at 37ºC.
- Spectrophotometer, thermostatable at 37ºC with a 540nm filter (530-550nm).

Procedure:

1. The working reagent and photometer (cuvette holder) were brought to 37 ºC.
2. Assay conditions:

   Wavelength : 540nm (530-550)

   Temperature : 37ºC

   Cuvette light path : 1cm.

3. The instrument was adjusted to zero with distilled water.
4. Pipetted into a cuvette:

   Working reagent (mL) - 1.0

   Calibrator - 1.0

5. The reagents were mixed and the absorbance (A_1) was read immediately and after 4 minutes of sample addition (A_2) was read.

Calculations:

The absorbance difference (A_2-A_1) of each point of the calibration curve was calculated and the values obtained were plotted against the CRP concentration of each calibrator dilution. CRP concentration in the sample was calculated by interpolation of its (A_2-A_1) in the calibration curve.

Detection Limit:

Values less than 0.05mg/L give non-reproducible results.

QUANTITATIVE DETERMINATION OF SALIVARY HAPTOGLOBIN BY IMMUNOTURBIDOMETRIC METHOD.[37]

Principle:

HAPTO is a quantitative turbidimetric test for the measurement of haptoglobin in human serum or plasma. Anti-haptoglobin antibodies when mixed with samples containing haptoglobin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the haptoglobin concentration of the patient sample, that can be quantified by comparison from a calibrator of know haptoglobin concentration.

Clinical Significance

The haptoglobin is an α_2-glycoprotein synthesized in the liver that binds hemoglobin irreversibly. The hapto-hemoglobin complexes, as well free haptoglobin itself, play significant roles in the iron storage and prevents of possible renal damage as a consequence of hemoglobin excretion. As an acute-phase protein, haptoglobin is increased in the presence of acute inflammatory process, tissue necrosis or malignancy.

Reagents:

Diluent (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.2. Sodium azide 0.95 g/L.
Antiserum (R2)	Goat serum, anti-human haptoglobin pH 7.5. Sodium azida 0.95 g/L.

Calibration Curve:

Prepared the following PROT CAL Calibrator dilutions in ClNa 9 g/L as diluent. Multiplied the concentration of the haptoglobin calibrator by the corresponding factor stated in table bellow to obtain the haptoglobin concentration of each dilution.

Calibrator Dilution	1	2	3	4	5	6
Calibrator (µL)	-	10	25	50	75	100
NaCl 9g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

Procedure:

1. Brought the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:

   Wavelength : 340 nm

   Temperature : 37 ºC

   Cuvette light path : 1cm
3. Adjusted the instrument to zero with distilled water.
4. Pipetted into a cuvette:

   Reagent R1 (µL) - 800 µL

   Sample or Calibrator (µL) - 10 µL
5. Mixed and read the absorbance (A_1) after the sample addition.
6. Immediately, pipetted into the cuvette:

   Reagent R2 (µL) - 200
7. Mixed and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Calculation:

The absorbance difference (A_2-A_1) of each point of the calibration curve was calculated and the values obtained were plotted against the haptoglobin concentration of each calibrator dilution. Haptoglobin concentration in the sample was calculated by interpolation of its (A_2-A_1) in the calibration curve.

Range: 30 – 200 mg/dL.

Detection Limit:

Values less than 1.3mg/dL give non-reproducible results.

Statistical Analysis:

Mean and standard deviation were estimated in the sample for each study group. Mean values were compared by using one-way ANOVA followed by multiple range tests by Tukey-HSD procedure.

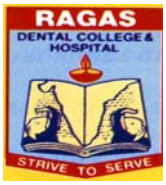
In the present study $P < 0.05$ was considered as the level of significance.

$$\text{Mean}(X) = \frac{\sum \overline{Xi}}{n}$$

$$\text{Standard Deviation} = \sqrt{\frac{\sum(\overline{Xi} - X)^2}{n-1}}$$

Where Xi is the individual observation and n is the sample size.

CASE SHEET PROFOMA



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DEPARTMENT OF ORAL MEDICINE RADIOLOGY

ESTIMATION OF SALIVARY IMMUNOGLOBULIN AND ACUTE PHASE PROTEINS IN PATIENTS WITH POTENTIALLY MALIGNANT DISORDERS, UNTREATED AND TREATED ORAL CANCER.

Serial no. Op. no.

Name: Age/ Sex:

Religion:

Occupation: Income:

Address: Phone no**:**

HABITS	**PRESENT**	**ABSENT**
Smoking		
Chewing		
Alcohol		

LESION	PRESENT	ABSENT
Potentially malignant disorders		
Untreated Oral Squamous Cell Carcinoma		
Treated Oral Squamous Cell carcinoma		

Date of sample collection:

PARAMETERS/ LESION	SALIVARY IgA (mg/l)	SALIVARY IgG (mg/l)	SALIVARY CRP (mg/l)	SALIVARY HAPTOGLOBIN (mg/l)
Potentially malignant disorders				
Untreated Oral Squamous Cell Carcinoma				
Treated Oral Squamous Cell carcinoma				

FIGURE 1: ARMAMENTARIUM FOR CLINICAL EXAMINATION

FIGURE 2: NORMAL MUCOSA

FIGURE 3: CLINICAL LESION - LEUKOPLAKIA

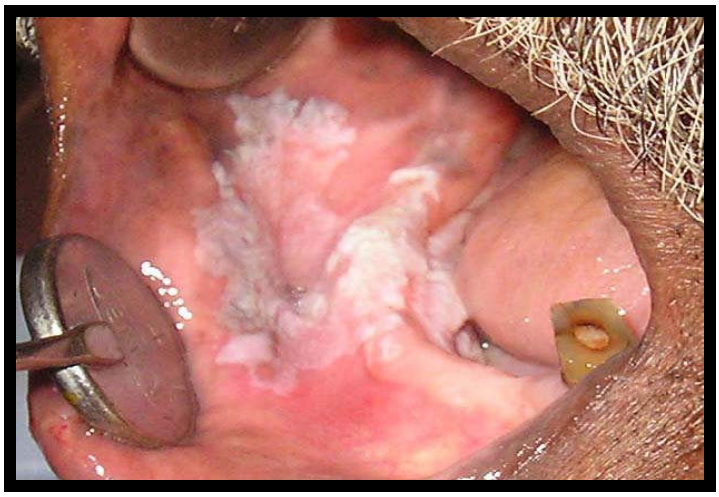


FIGURE 4: CLINICAL LESION – ORAL SUBMUCOUS FIBROSIS

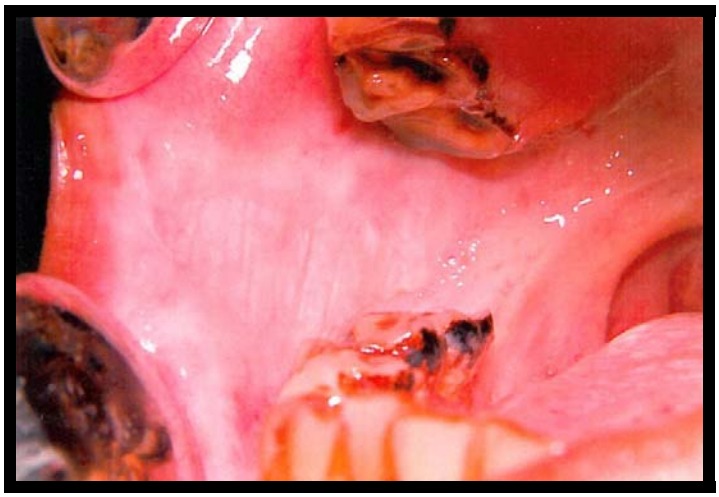


FIGURE 5: CLINICAL LESION – ORAL CANCER

FIGURE 6: CLINICAL LESION – ORAL CANCER

FIGURE 7: MATERIALS FOR SAMPLE COLLECTION

FIGURE 8: MATERIALS FOR BIOCHEMICAL ANALYSIS

FIGURE 9: SPECTROPHOTOMETER

FIGURE 10: ELISA READER AND WASHER

Table 1 and Graph 1 show Distribution of the subjects by Sex:

The study group consisted of a total number of 80 patients. Out of the 20 (100%), patients in Group I 18 (90%) were males and 2 (10%) were females. In Group II among the 20 (100%), subjects 15 (75%) were males and 5 (25%) females. In Group III among the 20 (100%), patients, 14(60%) were males and 6(40%) were females. Among the 20 (100%), subjects in Group IV, 15 (75%) were males and 5 (25%) were females. In total among the 80 (100%), 62 (77.5%) were males and 18 (22.5%) were females.

The sex wise distribution of subjects were found to be **statistically non significant**, which means that both the experimental and control subjects were similar with respect to sex in distribution with **p value 0.46.**

Table 2 and Graph 2 show Distribution of the subjects by Age:

The subjects were divided into six age groups which are as follows: 15-25 years, 26-35 years, 36-45 years, 46-55 years, 56-65 years and above 65 years. Among the 20 (100%), in group I, 5(25%) were between 15-25 years, 4(20%) between 26-35 years, 5(25%) were between 36-45 years, 2(10%) between 46-55years, 1(5%) between 56-65 years and 3(15%) were above 65 years. Among the 20 (100%), in group II none were in the age group of 15-25 years, 2(10%) between 26-35 years, 8(40%) were between 36-45 years, 4(20%) were between 46-55 years, 5(25%) were between 56-65 years and 1(5%) above 65 years. In group III, among the 20 (100%), 1(5%) belonged to 15-25 years, 3(15%) belonged to 26-35 years, 7(35%) belonged to 36-45 years, 4(20%) belonged to 46-55 years, 3(15%) belonged

to 56-65 years and 2(10%) belonged to age group of above 65 years. Among the 20 (100%), in group IV 3(15%) were in the age group of 15-25 years, 5(25%) between 26-35 years, 6(30%) were between 36-45 years, 2(10%) were between 46-55 years, 3(15%) were between 56-65 years and 1(5%) above 65 years. In total among the 80(100%), 9(11.25%) were between 15-25 years, 14(17.5%) between 26-35 years, 26(32.5%) were between 36-45 years, 12(15%) between 46-55years, 12(15%) between 56-65 years and 7(8.75%) were above 65 years. The age wise distribution of subjects were found to be **statistically not significant**, which means that both the experimental and control groups were similar with respect to age distribution with **p value 0.111**.

Table 3 and Graph 3 show Distribution of the subjects based on the habits:

The distributions of habits were grouped as follows, smoking, chewing, chewing and smoking, smoking and alcohol, chewing and alcohol, and chewing, smoking and alcohol.

In group I among the 20 (100%), subjects, 4(20%) had the habit of smoking, 8(40%) had the habit of chewing, 4(20%) had the habit of chewing and smoking while 3(15%) had the combined habits of smoking and alcohol consumption, 1(5%) had the habit of chewing and alcohol consumption and none had all three habits together.

In group II among the 20 (100%), subjects, 5(25%) had the habit of smoking, 7(35%) had the habit of chewing, 3(15%) had the habit of chewing

and smoking while 3(15%) had the combined habits of smoking and alcohol consumption, none had the habit of chewing and alcohol consumption and 2(10%) had all three habits together.

In group III out of the 20 (100%), subjects, 6(30%) had the habit of smoking, 6(30%) had the habit of chewing, 4(20%) had the habit of chewing and smoking, 3(15%) had the habit of smoking and alcohol consumption, none had the habit of chewing and alcohol consumption and 1(5%) had all three habits together.

In group IV among the 20 (100%), subjects, 11(55%) had no deleterious habits, 5(25%) had the habit of smoking, 2(10%) had the habit of chewing, 1(5%) had the habit of chewing and smoking, 1(5%) had the habit of smoking and alcohol consumption, none had the habit of chewing and alcohol consumption and none had all three habits together.

In total among the 80 (100%), 11(13.8%) had no deleterious habits, 20(25%) had the habit of smoking, 23(28.8%) had the habit of chewing, 12(15%) had the habit of chewing and smoking, 10(12.5%) had the habit of smoking and alcohol consumption, 1(1.3%) had the habit of chewing and alcohol consumption and 3(3.8%) had all three habits together. The distributions of subjects based on habits were found to be **statistically significant**, with the **p value 0.000**.

Table 4 and Graph 4 show Distribution of the subjects according to the lesion in group – I:

The subjects in Group – I were divided into two classes based on the type of potentially malignant disorder present as follows, Leukoplakia and Oral submucous fibrosis. Among the 20 (100%), subjects in Group I, 9(45%) had Oral submucous fibrosis and 11(55%) had Leukoplakia. The group is constant and hence the p-value is not attained.

Table 5 and Graph 5 show Distribution of the subjects according to the site of Leukoplakia in Group – I:

In Group I among the total of 20 (100%) subjects, 11(55%) had leukoplakia in which 7(63.6%) were present in the retro-commissure area, 2 (18.2%) in the buccal mucosa, 1(9.1%) in the tongue and 1(9.1%) in the floor of the mouth. The group is constant and hence p-value is not attained.

Table 6 and Graph 6 show Distribution of the subjects according to the grades of OSMF in Group – I:

In Group I 9(45%) subjects out of the total of 20(100%) had OSMF with 5(55.6%) in Grade III, 2(22.2%) in Grade I, 2(22.2%) in Grade IV and none in Grade II. The p-value is not attained as the group is constant.

Table 7 and Graph 7 show Distribution of the subjects according to the site of carcinoma in Groups – II and III:

In Groups II and III oral carcinoma was seen in 6 different sites: tongue, buccal mucosa, alveolar mucosa, floor of the mouth, palate and both in tongue and floor of the mouth.

In Group II among the 20 subjects, 5(25%) had carcinoma in the tongue, 8(40%) had in the buccal mucosa, 3(15%) had in the alveolar mucosa, 2(10%) had carcinoma in the floor of the mouth and 1(5%) in the palate and 1(5%) in both the tongue and the floor of the mouth.

In Group III among the 20 subjects, 6(30%) had carcinoma in the tongue, 9(45%) had in the buccal mucosa, 4(20%) had in the alveolar mucosa, none had carcinoma in the floor of the mouth and in the palate and 1(5%) had in both the tongue and the floor of the mouth.

In total among the 40 (100%), 11(27.5%) had carcinoma in the tongue, 17(42.5%) had in the buccal mucosa, 7(17.5%) had in the alveolar mucosa, 2(5%) had carcinoma in the floor of the mouth and 1(2.5%) in the palate and 2(5%) had in both the tongue and the floor of the mouth.The distribution of subjects according to the carcinoma site were found to be **statistically non significant**, with **p-value 0.655**.

Table 8 and Graph 8 show the Salivary IgA levels in Groups I, II, III and IV:

The maximum value for group I was 361.30 3µg/mL and the minimum value was 108.70 3µg/mL, in group II the maximum value was 380.30 3µg/mL and the minimum value was 124.403µg/mL, in group III 98.30 3µg/mL was the maximum value and 62.30 3µg/mL was the

minimum value and in group IV the maximum value was 109.20 3µg/mL and the minimum value was 32.60 3µg/mL.

Mean Salivary IgA was highest in patients with oral carcinoma (Group II) (253.35 ± 83.3µg/mL) followed by patients with potentially malignant disorders (Group I) (201.7 ± 76.01 µg/mL), then patients with treated oral carcinoma (Group III) (76.26 ± 9.20 µg/mL) and lowest in controls (Group IV) (72.87 ± 26.46 µg/mL). Comparison of these mean values by One-way ANOVA showed that there was a difference in mean values among the four study groups, with **p-value 0.000** which is significant.

Table 9 and Graph 9 show the Salivary IgG levels in Groups I, II, III and IV:

The maximum value for group I was 15.80 mg/dL and the minimum value was 1.30 mg/dL, in group II the maximum value was 68.30 mg/dL and the minimum value was 11.0 mg/dL, in group III 25.40 mg/dL was the maximum value and 13.80 mg/dL was the minimum value and in group IV the maximum value was 27.0 mg/dL and the minimum value was 1.3mg/dL.

Mean Salivary IgG was highest in patients with oral carcinoma (Group II) (33.86 ± 20.31mg/dL) followed by patients with treated oral carcinoma (Group III) (17.74 ± 3.12 µg/dL), then patients with potentially malignant disorders (Group I) (8.7± 4.07 µg/dL) and lowest in controls (Group IV) (6.58 ± 5.60 µg/dL). Comparison of these mean values by One-

way ANOVA showed that there was significant difference in mean values among the four study groups, with **p-value 0.000** which is significant.

Table 10 and Graph10 show the Salivary CRP levels in Groups I, II, III and IV:

The maximum value for group I was 0.1 mg/L and the minimum value was 0.0 mg/L, in group II the maximum value was 0.4 mg/L and the minimum value was 0.0 mg/L, in group III 0.4 mg/L was the maximum value and 0.0 mg/L was the minimum value and in group IV the maximum value was 0.5 mg/L and the minimum value was 0.0 mg/L.

Mean Salivary CRP was highest in patients with treated oral carcinoma (Group III) (0.120 ± 0.128mg/L) followed by controls (0.070 ± 0.13 mg/L), then patients with oral carcinoma (Group II) (0.050 ± 0.10 mg/L) and lowest in patients with potentially malignant disorders (Group I) (0.015± 0.036 µg/mL). Comparison of these mean values by One-way ANOVA showed that there was significant difference in mean values among the four study groups, with **p-value 0.021** which is significant.

Table 11 and Graph 11 show the Salivary Haptoglobin levels in Groups I, II, III and IV:

The maximum value for group I was 3.80 mg/dL and the minimum value was 0.6 mg/dL, in group II the maximum value was 7.4 mg/dL and the minimum value was 3.1 mg/dL, in group III 2.0mg/dL was the maximum value and 0.4 mg/dL was the minimum value and in group IV the maximum value was 4.0 mg/dL and the minimum value was 0.8 mg/dL.

Mean Salivary Haptoglobin was highest in patients with oral carcinoma (Group II) (4.56 ± 1.19mg/dL) followed by controls (Group IV) (1.91 ± 0.895 mg/dL), then patients with potentially malignant disorders (Group I) (1.43 ± 0.843 mg/dL) and lowest in patients with treated oral carcinoma (Group III) (1.285 ± 0.492 mg/dL). Comparison of these mean values by One-way ANOVA showed that there was significant difference in mean values among the four study groups, with **p-value 0.000** which is significant.

Table 12 and Graph 12 show the comparision of Salivary IgA levels in Group I with Groups II, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgA levels between Group I and Group II of (-51.6 ± 18.4) with p-value of 0.031 that is statistically significant. There is a significant difference in the mean salivary IgA levels between Group I and Group III of (125.4 ± 18.4) with p-value of 0.000. The mean difference in the Salivary IgA levels between Group I and IV is (128.8 ± 18.4) that is statistically significant with a **p-value of 0.000.**

Table 13 and Graph 13 show the comparison of the Salivary IgA levels in Group II with Groups I, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgA levels between Group II and Group I of (51.6 ± 18.4) with p-

value of 0.031 that is statistically significant. There is a significant difference in the mean salivary IgA levels between Group II and Group III of (177.1 ± 18.4) with p-value of 0.000. The mean difference in the Salivary IgA levels between Group II and IV is (180.5 ± 18.4) that is statistically significant with a **p-value of 0.000.**

Table 14 and Graph 14 show the comparison of Salivary IgA levels in Group III with Groups I, II and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgA levels between Group III and Group I of (125.4 ± 18.4) with p-value of 0.000 that is statistically significant. There is a significant difference in the mean salivary IgA levels between Group III and Group II of (-177.1 ± 18.4) with p-value of 0.000. The mean difference in the Salivary IgA levels between Group III and IV is (3.4 ± 18.4) that is statistically not significant with a **p-value of 0.998.**

Table 15 and Graph 15 show the comparison of the Salivary IgA levels in Group IV with Groups I, II and III:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgA levels between Group IV and Group I of (-128.8 ± 18.4) with p-value of 0.000 that is statistically significant. There is a significant difference in the mean salivary IgA levels between Group IV and Group II of (-180.5 ± 18.4) with p-value of 0.000. The mean difference in the

Salivary IgA levels between Group IV and III is (-3.4 ± 18.4) that is statistically not significant with a **p-value of 0.998.**

Table 16 and Graph 16 show the comparison of Salivary IgG levels in Group I with Groups II, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgG levels between Group I and Group II of (-25.16 ± 3.43) with p-value of 0.000 that is statistically significant. There is a significant difference in the mean salivary IgG levels between Group I and Group III of (-9.04 ± 3.43) with p-value of 0.049. The mean difference in the Salivary IgG levels between Group I and IV is (2.11 ± 3.43) that is statistically not significant with a **p-value of 0.927.**

Table 17 and Graph 17 show the comparison of Salivary IgG levels in Group II with Groups I, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgG levels between Group II and Group I of (25.16 ± 3.43) with p-value of 0.000 that is statistically significant. There is a significant difference in the mean salivary IgG levels between Group II and Group III of (16.1 ± 3.43) with p-value of 0.000. The mean difference in the Salivary IgG levels between Group II and IV is (27.2 ± 3.43) that is statistically significant with a **p-value of 0.000.**

Table 18 and Graph 18 show the comparison of Salivary IgG in Group III with Groups I, II and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgG levels between Group III and Group I of (9.04 ± 3.43) with p-value of 0.049 that is statistically significant.

There is a significant difference in the mean salivary IgG levels between Group III and Group II of (-16.1 ± 3.43) with p-value of 0.000. The mean difference in the Salivary IgG levels between Group III and IV is (11.1 ± 3.43) that is statistically significant with a **p-value of 0.009.**

Table 19 and Graph 19 show the comparison of Salivary IgG in Group IV with Groups I, II and III:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary IgG levels between Group IV and Group I of (-2.1 ± 3.43) with p-value of 0.927 that is statistically not significant. There is a significant difference in the mean salivary IgG levels between Group IV and Group II of (-27.2 ± 3.43) with p-value of 0.000. The mean difference in the Salivary IgG levels between Group IV and III is (-11.1 ± 3.43) that is statistically significant with a **p-value of 0.009.**

Table 20 and Graph 20 show the comparison of Salivary CRP levels in Group I with Groups II, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that the mean difference in the Salivary CRP levels between Group I and Group II of (-0.35 ± 0.033) with p-value of 0.723 that is statistically not significant. There is a significant difference in the mean salivary CRP levels between Group I and Group III of (-1.050 ± 0.033) with p-value of 0.013. The mean difference in the Salivary CRP levels between Group I and IV is (-0.055 ± 0.033) that is statistically not significant with a **p-value of 0.360.**

Table 21 and Graph 21 show the comparison of Salivary CRP levels in Group II with Groups I, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed the mean difference in the Salivary CRP levels between Group II and Group I of (0.035 ± 0.033) with p-value of 0.723 that is statistically not significant. The mean difference in the salivary CRP levels between Group II and Group III is (-0.070 ± 0.033) with p-value of 0.164 that is statistically not significant. The mean difference in the Salivary CRP levels between Group II and IV is (-0.020 ± 0.033) that is statistically not significant with a **p-value of 0.932.**

Table 22 and Graph 22 show the comparison of Salivary CRP in Group III with Groups I, II and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary CRP levels between Group III and Group I of (-1.050 ± 0.033) with p-value of 0.013 that is statistically significant. The mean difference in the salivary CRP levels between Group III and Group II is (-0.070 ± 0.033) with p-value of 0.164 which is statistically not significant. The mean difference in the Salivary CRP levels between Group III and IV is (0.050 ± 0.033) that is statistically not significant with a **p-value of 0.445.**

Table 23 and Graph 23 show the comparison of Salivary CRP in Group IV with Groups I, II and III:

The comparison between the groups had been done by following Tukey HSD procedure which showed the mean difference in the Salivary CRP levels between Group IV and Group I of (0.055 ± 0.033) with p-value of 0.360 that is statistically not significant. The mean difference in the salivary CRP levels between Group IV and Group II is (0.020 ± 0.033) with p-value of 0.932. The mean difference in the Salivary CRP levels between Group IV and III is (0,050 ± 0.033) that is statistically not significant with a **p-value of 0.445.**

Table 24 and Graph 24 show the comparison of Salivary Haptoglobin levels in Group I with Groups II, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary Haptoglobin levels between Group I and Group II of (-3.135 ± 0.282) with p-value of 0.000 that is statistically significant. The mean difference in the salivary Haptoglobin levels between Group I and Group III is (0.145 ± 0.282) with p-value of 0.955 which is statistically not significant. The mean difference in the Salivary Haptoglobin levels between Group I and IV is (-0.48 ± 0.282) that is statistically not significant with a **p-value of 0.329.**

Table 25 and Graph 25 show the comparison of Salivary Haptoglobin levels in Group II with Groups I, III and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed that there is a mean difference in the Salivary Haptoglobin levels between Group II and Group I of (3.135 ± 0.282) with p-value of 0.000 that is statistically significant. There is a significant difference in the mean salivary Haptoglobin levels between Group II and Group III of (3.28 ± 0.282) with p-value of 0.000. The mean difference in the Salivary Haptoglobin levels between Group II and IV is (2.65 ± 0.282) that is statistically significant with a **p-value of 0.000.**

Table 26 and Graph 26 show the comparison of Salivary Haptoglobin in Group III with Groups I, II and IV:

The comparison between the groups had been done by following Tukey HSD procedure which showed the mean difference in the Salivary Haptoglobin levels between Group III and Group I of (-0.145 ± 0.282) with p-value of 0.955 that is statistically not significant. There is a significant difference in the mean salivary Haptoglobin levels between Group III and Group II of (-3.28 ± 0.282) with p-value of 0.000. The mean difference in the Salivary Haptoglobin levels between Group III and IV is (-0.625 ± 0.282) that is statistically not significant with a **p-value of 0.128.**

Table 27 and Graph 27 show the comparison of Salivary Haptoglobin in Group IV with Groups I, II and III:

The comparison between the groups had been done by following Tukey HSD procedure which showed the mean difference in the Salivary Haptoglobin levels between Group IV and Group I of (0.48 ± 0.282) with p-value of 0.329 that is statistically not significant. There is a significant difference in the mean salivary Haptoglobin levels between Group IV and Group II of (-2.655 ± 0.282) with p-value of 0.000. The mean difference in the Salivary Haptoglobin levels between Group IV and III is (0.625 ± 0.282) that is statistically not significant with a **p-value of 0.128.**

TABLE – I: DISTRIBUTION OF SUBJECTS BY SEX

SEX	GROUP I (Potentially malignant disorders)		GROUP II (Untreated oral carcinoma)		GROUP III (Treated oral carcinoma)		GROUP IV (Controls)		TOTAL	
MALE	18	90%	15	75%	14	60%	15	75%	62	77.5%
FEMALE	2	10%	5	25%	6	40%	5	25%	18	22.5%
TOTAL	20	100%	20	100%	20	100%	20	100%	80	100%

P-value: 0.46 (not significant)

TABLE – II: DISTRIBUTION OF SUBJECTS BY AGE

AGE (Years)	GROUP I (Potentially malignant disorders)		GROUP II (Untreated oral carcinoma)		GROUP III (Treated oral carcinoma)		GROUP IV (Controls)		TOTAL	
15-25	5	25%	0	0%	1	5%	3	15%	9	11.25%
26 – 35	4	20%	2	10%	3	15%	5	25%	14	17.5%
36 – 45	5	25%	8	40%	7	35%	6	30%	26	32.5%
46 – 55	2	10%	4	20%	4	20%	2	10%	12	15%
56 – 65	1	5%	5	25%	3	15%	3	15%	12	15%
>65	3	15%	1	5%	2	10%	1	5%	7	8.75%
TOTAL	20	100%	20	100%	20	100%	20	100%	80	100%

P-value: 0.111(not significant)

TABLE – III: DISTRIBUTION OF SUBJECTS BASED ON HABITS

HABIT/ GROUP	No habits		Smoking		Chewing		Chewing + Smoking		Smoking + Alcohol		Chewing + Alcohol		Chewing + Smoking + Alcohol		Total	
Group I	0	0%	4	20%	8	40%	4	20%	3	15%	1	5%	0	0%	20	100%
Group II	0	0%	5	25%	7	35%	3	15%	3	15%	0	0%	2	10%	20	100%
Group III	0	0%	6	30%	6	30%	4	20%	3	15%	0	0%	1	5%	20	100%
Group IV	11	55%	5	25%	2	10%	1	%	1	5%	0	0%	0	0%	20	100%
Total	1	13.8%	20	25%	23	28.8%	12	15%	10	12.5%	1	1.3%	3	3.8%	80	100%

P-value: 0.000 (significant)

TABLE – IV DISTRIBUTION OF SUBJECTS ACCORDING TO LESION

		GROUP – I		TOTAL	
LESION	OSMF	9	45%	9	45%
	LEUKOPLAKIA	11	55%	11	55%
TOTAL		20	100%	20	100%

TABLE – V DISTRIBUTION OF SUBJECTS ACCORDING TO THE SITE OF LEUKOPLAKIA

		GROUP – I		TOTAL	
LEUKOPLAKIA SITE	RETRO COMMISSURE AREA	7	63.6%	7	63.6%
	BUCCAL MUCOSA	2	18.2%	2	18.2%
	FLOOR OF THE MOUTH	1	9.1%	1	9.1%
	TONGUE	1	9.1%	1	9.1%
TOTAL		11	100%	11	100%

TABLE – VI DISTRIBUTION OF SUBJECTS ACCORDING TO THE GRADE OF ORAL SUBMUCOUS FIBROSIS

		GROUP – I		TOTAL	
OSMF GRADE	GRADE I	2	22.2%	2	22.2%
	GRADE II	0	0%	0	0%
	GRADE III	5	55.6%	5	55.6%
	GRADE IV	2	22.2%	2	22.2%
TOTAL		9	100%	9	100%

TABLE – VII DISTRIBUTION OF SUBJECTS ACCORDING TO THE SITE OF CARCINOMA

		GROUPS				TOTAL	
		GROUP – II		GROUP – III			
CARCINOMA SITE	TONGUE	5	25%	6	30%	11	7.5%
	BUCCAL MUCOSA	8	40%	9	45%	17	42.5%
	ALVEOLAR MUCOSA	3	15%	4	20%	7	17.5%
	FLOOR OF THE MOUTH	2	10%	0	0%	2	5%
	PALATE	1	5%	0	0%	1	2.5%
	TONGUE + FLOOR OF THE MOUTH	1	5%	1	5%	2	5%

TOTAL	20	100%	20	100%	40	100%

P-Value: 0.655 (not significant)

TABLE – VIII SALIVARY IgA LEVELS IN GROUP I, II, III AND IV

S.NO	GROUP – I µg/mL	GROUP – II µg/mL	GROUP – III µg/mL	GROUP – IV µg/mL
1	132.92	141.44	67.45	88.21
2	156.24	125.71	82.0	107
3	287.8	338.98	62.3	109.2
4	317.10	380.34	73.4	47.6
5	113.76	273.73	66.0	43.7
6	150.84	124.42	72.65	33.3
7	142.63	139.23	79.78	79
8	243.69	268.23	64.5	40.3
9	133.9	246.09	79.8	32.6
10	161.55	330.23	68.4	43.3
11	180.6	208.66	78.4	40.6
12	113.17	237.33	89.0	83.6
13	230.74	250.42	98.3	103
14	237.13	273.08	83.7	82.7
15	241.6	356.07	79.2	74.84
16	108.79	365.78	77.8	103.85
17	161	295.74	75.28	87.78
18	296.6	153.45	87.8	81.67

19	361.37	234.46	72.8	81.2
20	262.8	324.64	67	94.24

P-Value: 0.000 (significant)

TABLE – IX SALIVARY IgG LEVELS IN GROUP I, II, III AND IV

S.NO	GROUP – I mg/dL	GROUP – II mg/dL	GROUP – III mg/dL	GROUP – IV mg/dL
1	7.6	22	25.4	27.9
2	1.3	15.2	16.7	4.8
3	14.7	20	15.4	6.0
4	13.0	11	18.5	8.2
5	5.5	29	16	5.5
6	15.8	55.8	25.0	5.4
7	15.0	12.0	16.5	6.7
8	5.3	14.2	17.5	5.6
9	6.9	23.3	14.6	6.5
10	15.3	18.8	13.8	3.4
11	9.6	4.1	17.4	3.7
12	6.4	53.4	17.57	1.5
13	5.5	57.0	18.4	3.8
14	5.3	68.3	21.30	5.5
15	9.2	55.0	20.2	10.3
16	9.2	48.6	15.0	2.8
17	5.9	52.3	17.6	7.2
18	8.1	66	15.0	1.3

19	8.9	14.5	16.3	3.0
20	5.5	25.7	16.7	13.5

P-Value: 0.000(significant)

TABLE – X SALIVARY CRP LEVELS IN GROUP I, II, III AND IV

S.NO	GROUP – I mg/L	GROUP – II mg/L	GROUP – III mg/L	GROUP – IV mg/L
1	0.1	0.1	0	0.5
2	0	0	0	0
3	0	0	0.1	0.1
4	0	0	0.1	0.1
5	0	0.2	0.4	0
6	0	0.1	0.2	0.1
7	0	0	0.1	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0.2	0
11	0	0	0.1	0
12	0	0	0.1	0.3
13	0	0	0.4	0
14	0	0	0.2	0.2
15	0	0.4	0.1	0
16	0	0.1	0	0
17	0	0	0	0
18	0.1	0	0.1	0

19	0	0.1	0.3	0.1
20	0.1	0	0	0

P-Value:0.021(significant)

TABLE-XI SALIVARY HAPTOGLOBIN LEVELS IN GROUP I, II, III & IV

S.NO	GROUP – I mg/dL	GROUP – II mg/dL	GROUP – III mg/dL	GROUP – IV mg/dL
1	1.1	6.2	1.4	0.8
2	2.8	3.7	2.2	1.7
3	2.4	3.5	1.5	2.0
4	3.8	3.5	0.4	1.2
5	1.6	3.1	0.7	1.3
6	2.4	5.0	1.8	2.2
7	1.8	3.3	2	1.5
8	0.6	4.4	1.4	1.0
9	0.7	4.1	0.8	1.3
10	1.2	5.5	0.5	2.2
11	1.0	3.9	1.7	2.7
12	1.2	4.1	1.5	1.7
13	0.7	3.6	1.0	0.9
14	0.9	7.4	1.8	4
15	0.7	6.3	1.3	3.3
16	0.8	5.8	1.6	3.5
17	0.8	3.7	1.4	1.0
18	1.1	5.5	1.8	1.9

19	1.7	3.9	1.5	1.7
20	1.3	4.8	0.4	2.3

P-Value: 0.000(significant)

TABLE – XII COMPARISON OF SALIVARY IgA LEVELS OF GROUP – I WITH GROUPS – II, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P - VALUE
GROUP – I POTENTIALLY MALIGNANT DISORDERS	GROUP – II ORAL CARCINOMA	-51.6	18.4	0.031
	GROUP – III TREATED ORAL CARCINOMA	125.4	18.4	0.000
	GROUP – IV CONTROLS	128.8	18.4	0.000

TABLE – XIII COMPARISON OF SALIVARY IgA LEVELS OF GROUP – II WITH GROUPS – I, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – II ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	51.6	18.4	0.031
	GROUP – III TREATED ORAL CARCINOMA	177.1	18.4	0.000

	GROUP – IV CONTROLS	180.5	18.4	0.000

TABLE – XIV COMPARISON OF SALIVARY IgA LEVELS OF GROUP – III WITH GROUPS – I, II AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – III TREATED ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	125.4	18.4	0.000
	GROUP – II ORAL CARCINOMA	-177.1	18.4	0.000
	GROUP – IV CONTROLS	3.4	18.4	0.998

TABLE – XV COMPARISON OF SALIVARY IgA LEVELS OF GROUP – IV WITH GROUPS – I, II AND III

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – IV CONTROLS	GROUP – I POTENTIALLY MALIGNANT DISORDERS	-128.8	18.4	0.000
	GROUP – II ORAL CARCINOMA	-180.5	18.4	0.000

	GROUP – III TREATED ORAL CARCINOMA	-3.4	18.4	0.998

TABLE – XVI COMPARISON OF SALIVARY IgG LEVELS OF GROUP – I WITH GROUPS – II, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P - VALUE
GROUP – I POTENTIALLY MALIGNANT DISORDERS	GROUP – II ORAL CARCINOMA	-25.16	3.43	0.000
	GROUP – III TREATED ORAL CARCINOMA	-9.04	3.43	0.049
	GROUP – IV CONTROLS	2.11	3.43	0.927

TABLE – XVII COMPARISON OF SALIVARY IgG LEVELS OF GROUP – II WITH GROUPS – I, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – II ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	25.16	3.43	0.000
	GROUP – III TREATED ORAL CARCINOMA	16.1	3.43	0.000

	GROUP – IV CONTROLS	27.2	3.43	0.000

TABLE – XVIII COMPARISON OF SALIVARY IgG LEVELS OF GROUP – III WITH GROUPS – I, II AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – III TREATED ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	9.04	3.43	0.049
	GROUP – II ORAL CARCINOMA	-16.1	3.43	0.000
	GROUP – IV CONTROLS	11.1	3.43	0.009

TABLE – XIX COMPARISON OF SALIVARY IgG LEVELS OF GROUP – IV WITH GROUPS – I, II AND III

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – IV CONTROLS	GROUP – I POTENTIALLY MALIGNANT DISORDERS	-2.1	3.43	0,927
	GROUP – II ORAL CARCINOMA	-27.2	3.43	0.000

	GROUP – III TREATED ORAL CARCINOMA	-11.1	3.43	0.009

TABLE – XX COMPARISON OF SALIVARY CRP LEVELS OF GROUP – I WITH GROUPS – II, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P - VALUE
GROUP – I POTENTIALLY MALIGNANT DISORDERS	GROUP – II ORAL CARCINOMA	-0.35	0.033	0.723
	GROUP – III TREATED ORAL CARCINOMA	-1.050	0.033	0.013
	GROUP – IV CONTROLS	-0.055	0.033	0.360

TABLE – XXI COMPARISON OF SALIVARY CRP LEVELS OF GROUP – II WITH GROUPS – I, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – II ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	0.035	0.033	0.723
	GROUP – III TREATED ORAL CARCINOMA	-0.070	0.033	0.164

	GROUP – IV CONTROLS	-0.020	0.033	0.932

TABLE – XXII COMPARISON OF SALIVARY CRP LEVELS OF GROUP – III WITH GROUPS – I, II AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – III TREATED ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	-1.050	0.033	0.013
	GROUP – II ORAL CARCINOMA	-0.070	0.033	0.164
	GROUP – IV CONTROLS	0.050	0.033	0.445

TABLE – XXIII COMPARISON OF SALIVARY CRP LEVELS OF GROUP – IV WITH GROUPS – I, II AND III

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – IV CONTROLS	GROUP – I POTENTIALLY MALIGNANT DISORDERS	0.055	0.033	0.360
	GROUP – II ORAL CARCINOMA	0.020	0.033	0.932

	GROUP – III TREATED ORAL CARCINOMA	0.050	0.033	0.445

TABLE – XXIV COMPARISON OF SALIVARY HAPTOGLOBIN LEVELS OF GROUP – I WITH GROUPS – II, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P - VALUE
GROUP – I POTENTIALLY MALIGNANT DISORDERS	GROUP – II ORAL CARCINOMA	-3.135	0.282	0.000
	GROUP – III TREATED ORAL CARCINOMA	0.145	0.282	0.955
	GROUP – IV CONTROLS	-0.48	0.282	0.329

TABLE – XXV COMPARISON OF SALIVARY HAPTOGLOBIN LEVELS OF GROUP – II WITH GROUPS – I, III AND IV

GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – II ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	3.135	0.282	0.000
	GROUP – III TREATED ORAL	3.28	0.282	0.000

	CARCINOMA			
	GROUP – IV CONTROLS	2.65	0.282	0.000

TABLE – XXVI COMPARISON OF SALIVARY HAPTOGLOBIN LEVELS OF GROUP – III WITH GROUPS – I, II AND IV

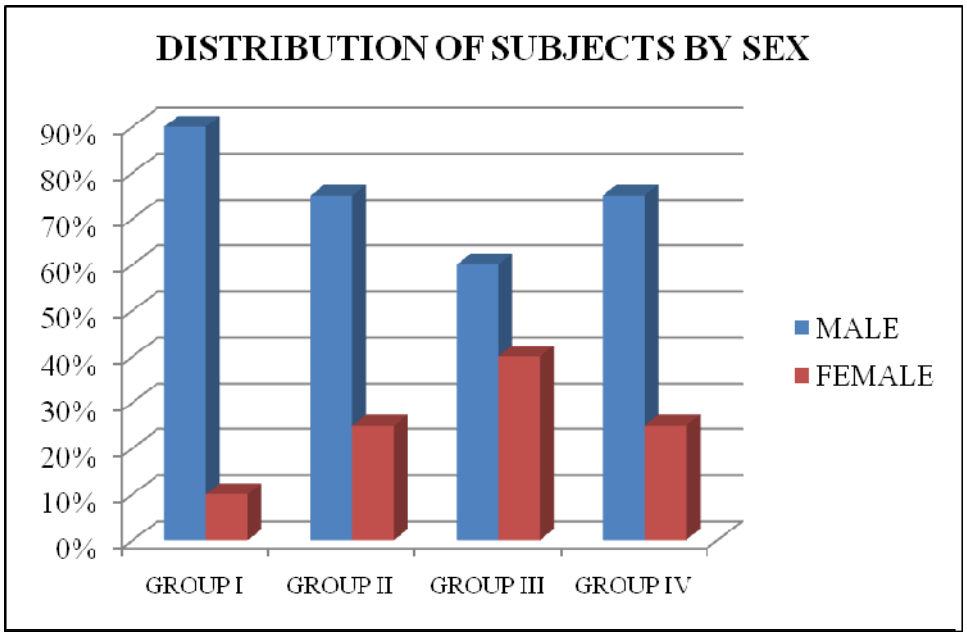
GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – III TREATED ORAL CARCINOMA	GROUP – I POTENTIALLY MALIGNANT DISORDERS	-0.145	0.282	0.955
	GROUP – II ORAL CARCINOMA	-3.28	0.282	0.000
	GROUP – IV CONTROLS	-0.625	0.282	0.128

TABLE – XXVII COMPARISON OF SALIVARY HAPTOGLOBIN LEVELS OF GROUP – IV WITH GROUPS – I, II AND III

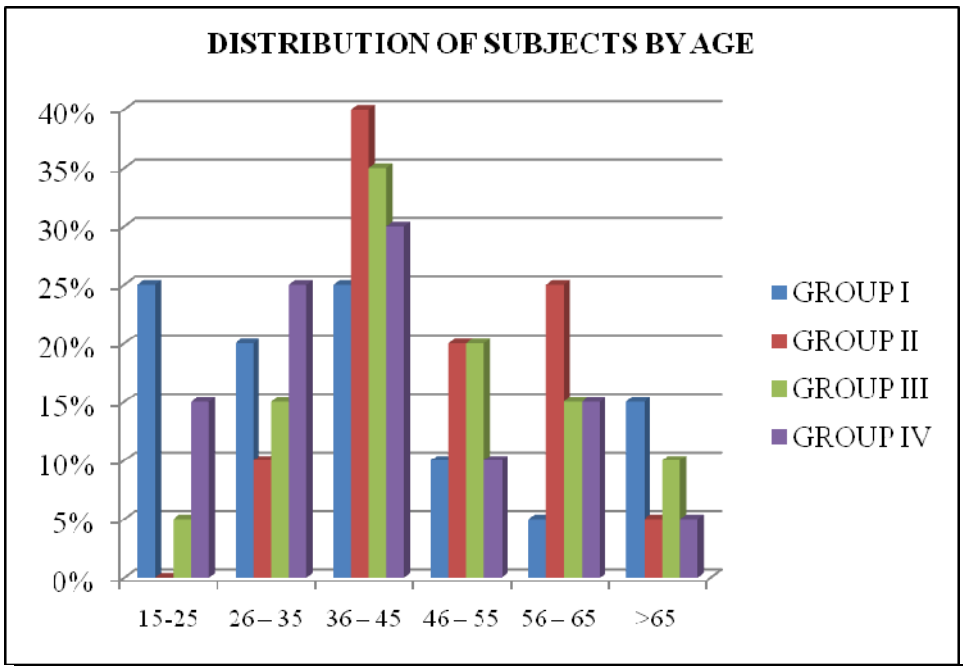
GROUP	COMPARISON GROUPS	MEAN DIFFERENCE	STANDARD ERROR	P – VALUE
GROUP – IV CONTROLS	GROUP – I POTENTIALLY MALIGNANT DISORDERS	0.48	0.282	0.329
	GROUP – II ORAL	-2.655	0.282	0.000

	CARCINOMA			
	GROUP – III TREATED ORAL CARCINOMA	0.625	0.282	0.128

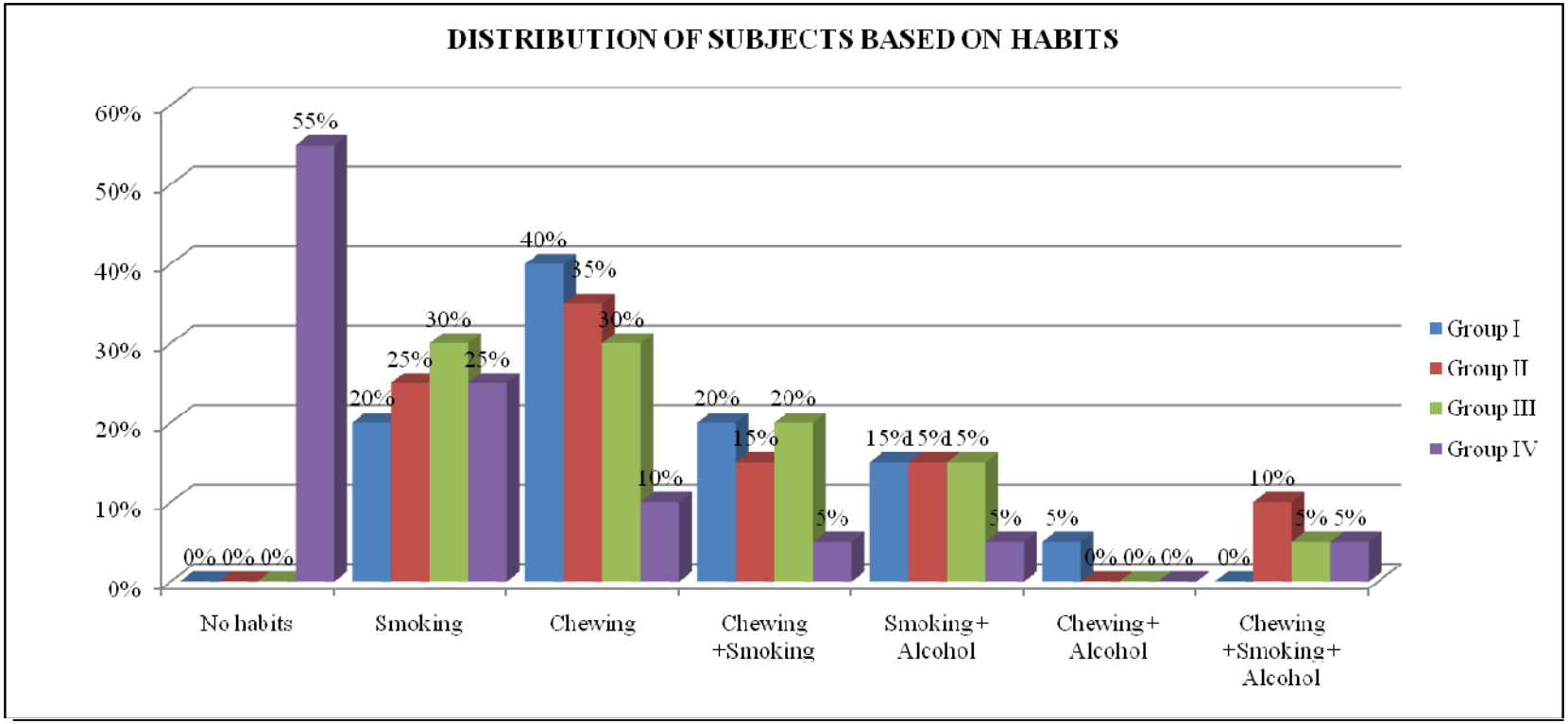
GRAPH -I



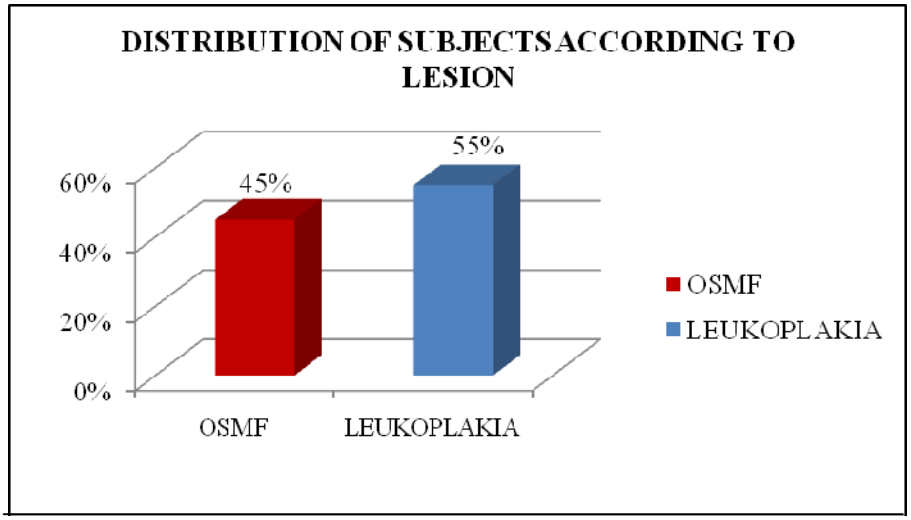
GRAPH – II



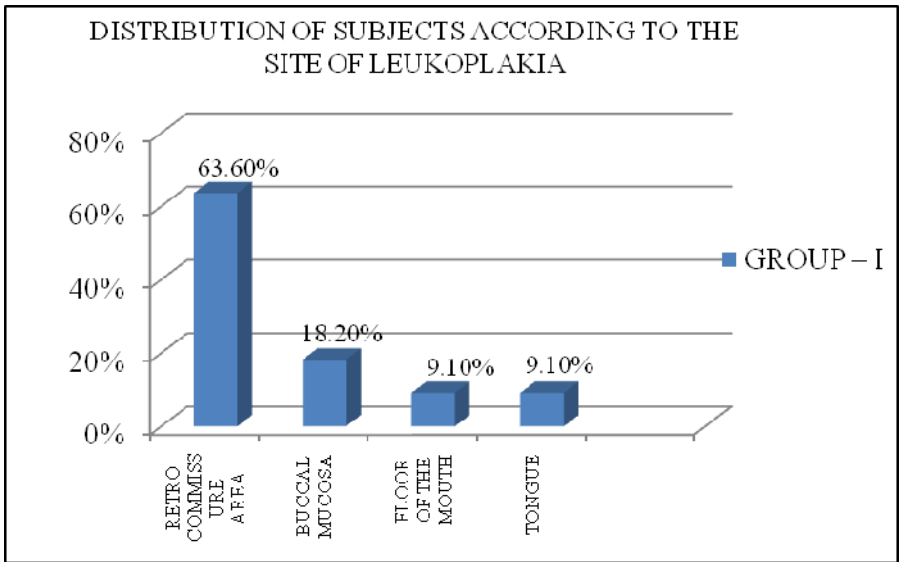
GRAPH – III


DISTRIBUTION OF SUBJECTS BASED ON HABITS
60%
50%
40%
30%
20%
10%
0%
55%
0% 0% 0%
20%
25%
30%
25%
40%
35%
30%
10%
20%
15%
20%
5%
15%
15%
15%
5%
5%
0% 0% 0%
0%
10%
5% 5%
No habits
Smoking
Chewing
Chewing +Smoking
Smoking+ Alcohol
Chewing+ Alcohol
Chewing +Smoking+ Alcohol
Group I
Group II
Group III
Group IV

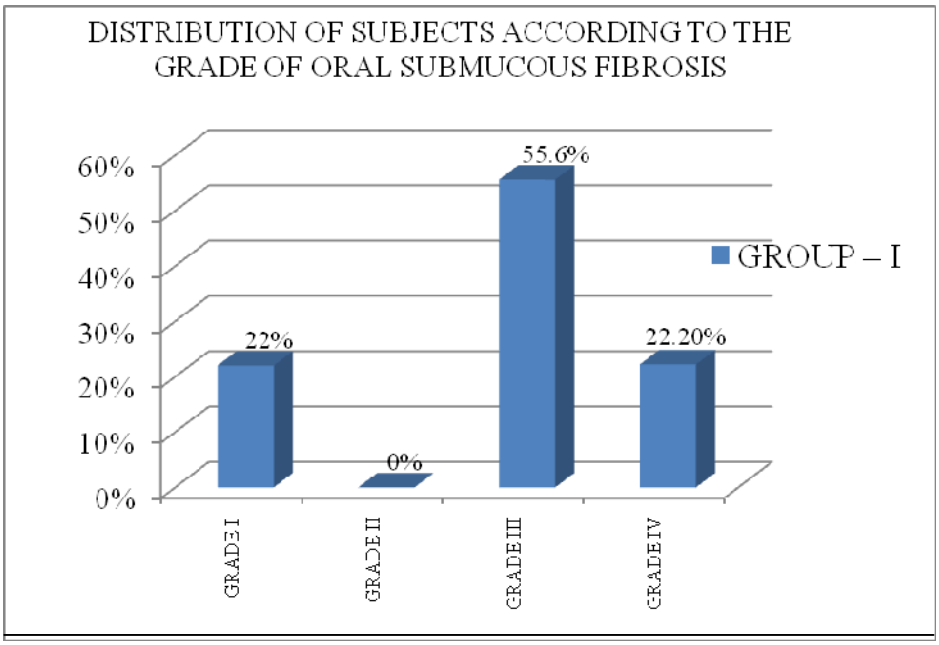
GRAPH – IV



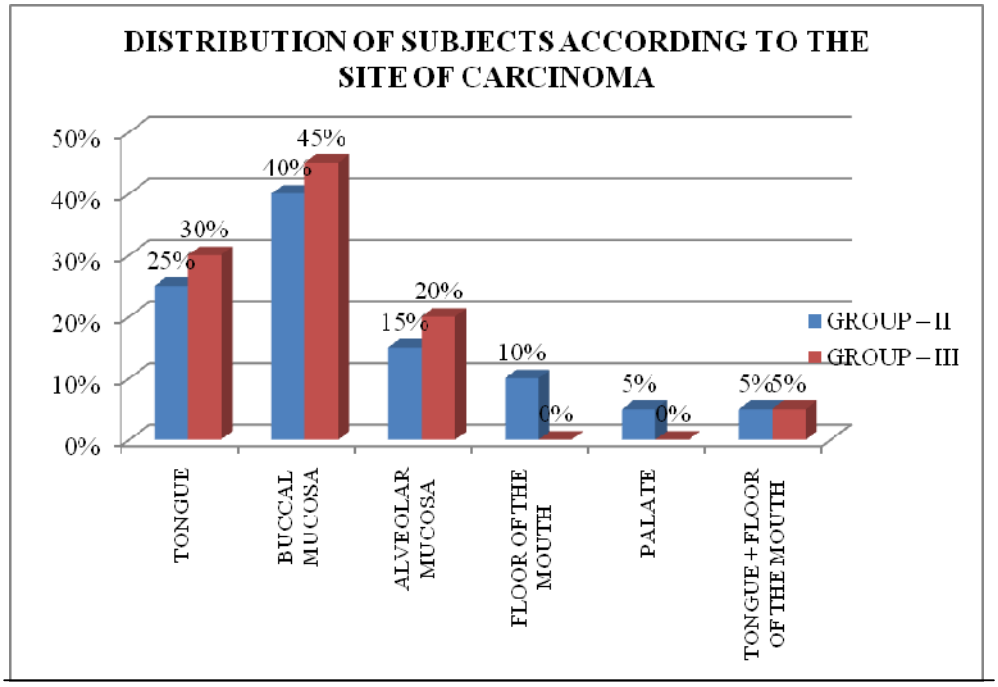
GRAPH – V



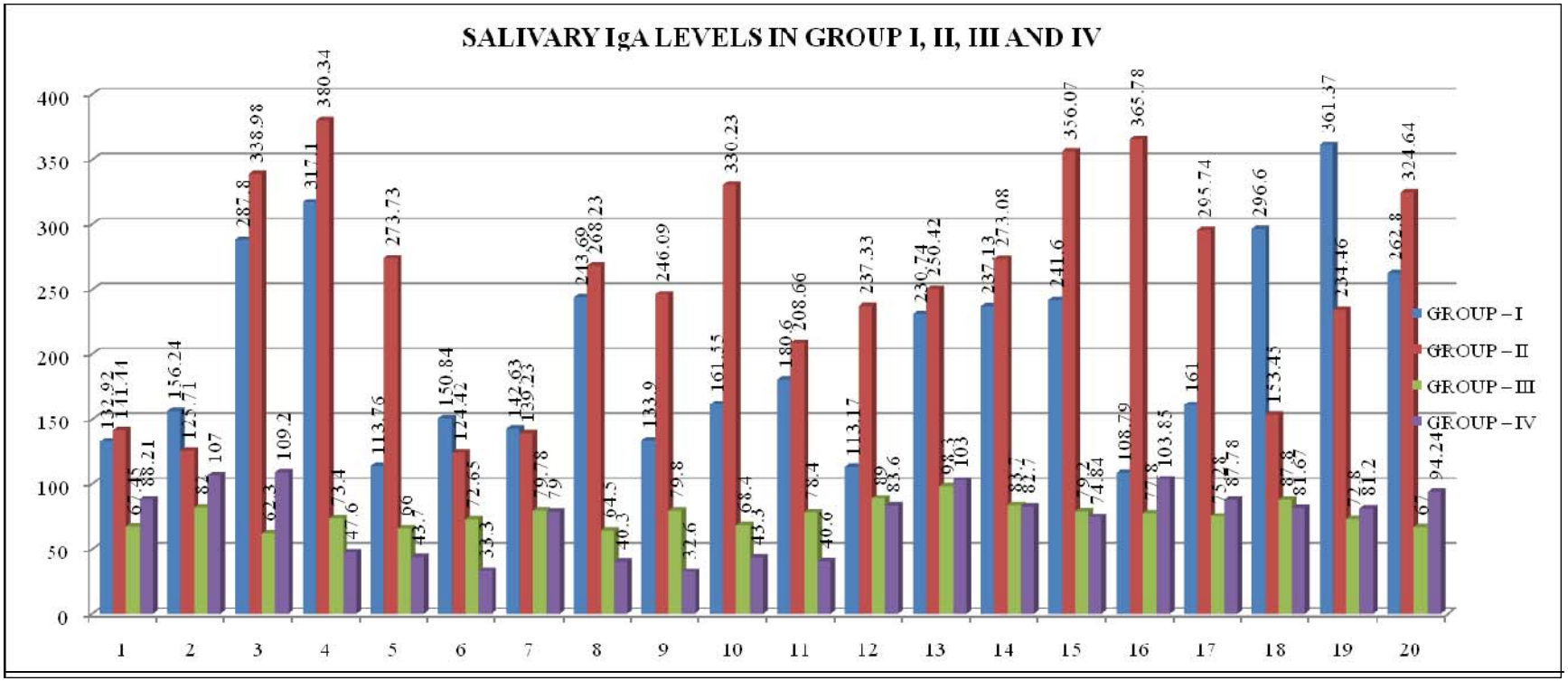
GRAPH – VI



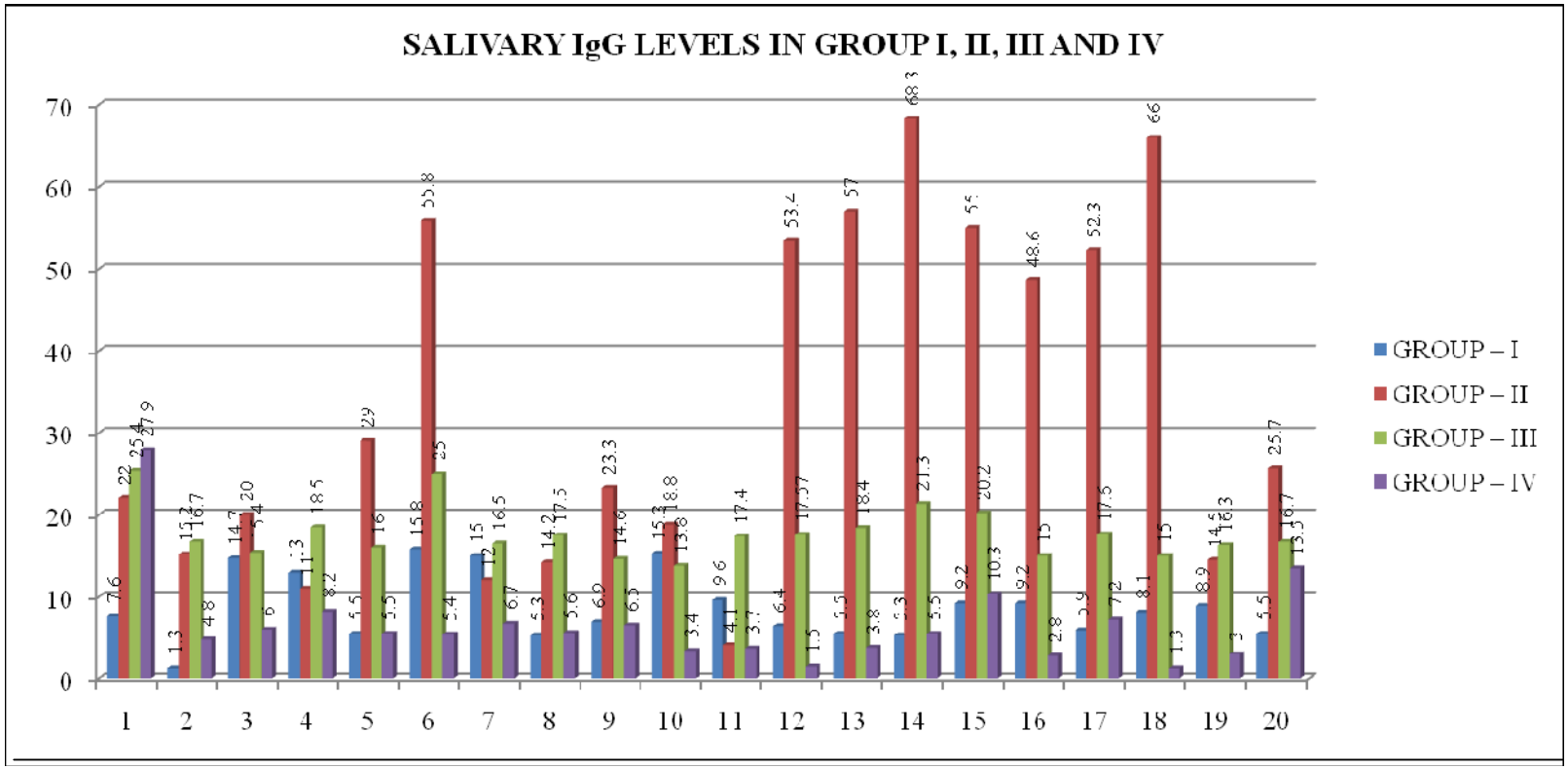
GRAPH – VII



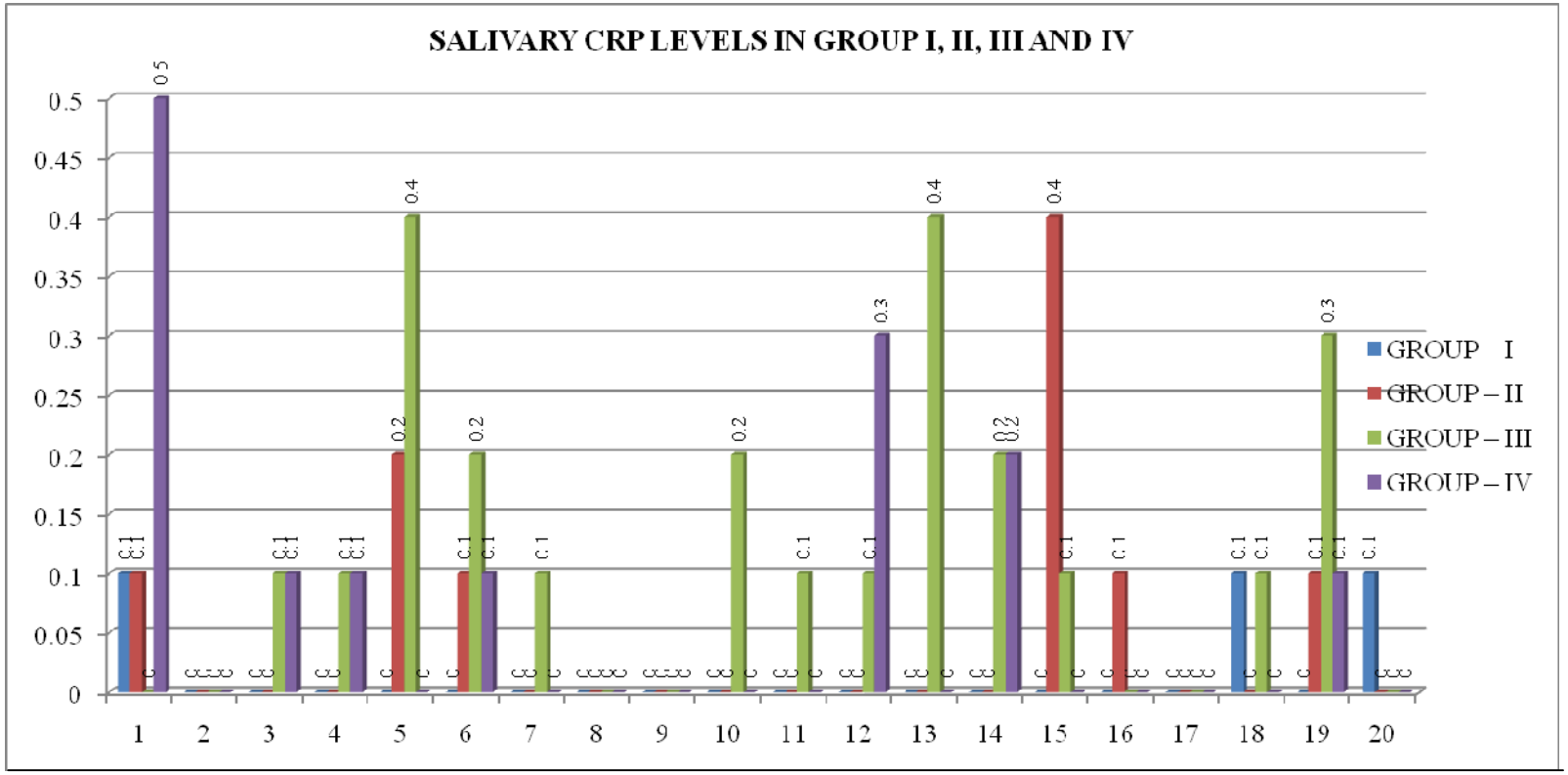
GRAPH – VIII


SALIVARY IgA LEVELS IN GROUP I, II, III AND IV
400
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
GROUP – I
GROUP – II
GROUP – III
GROUP – IV

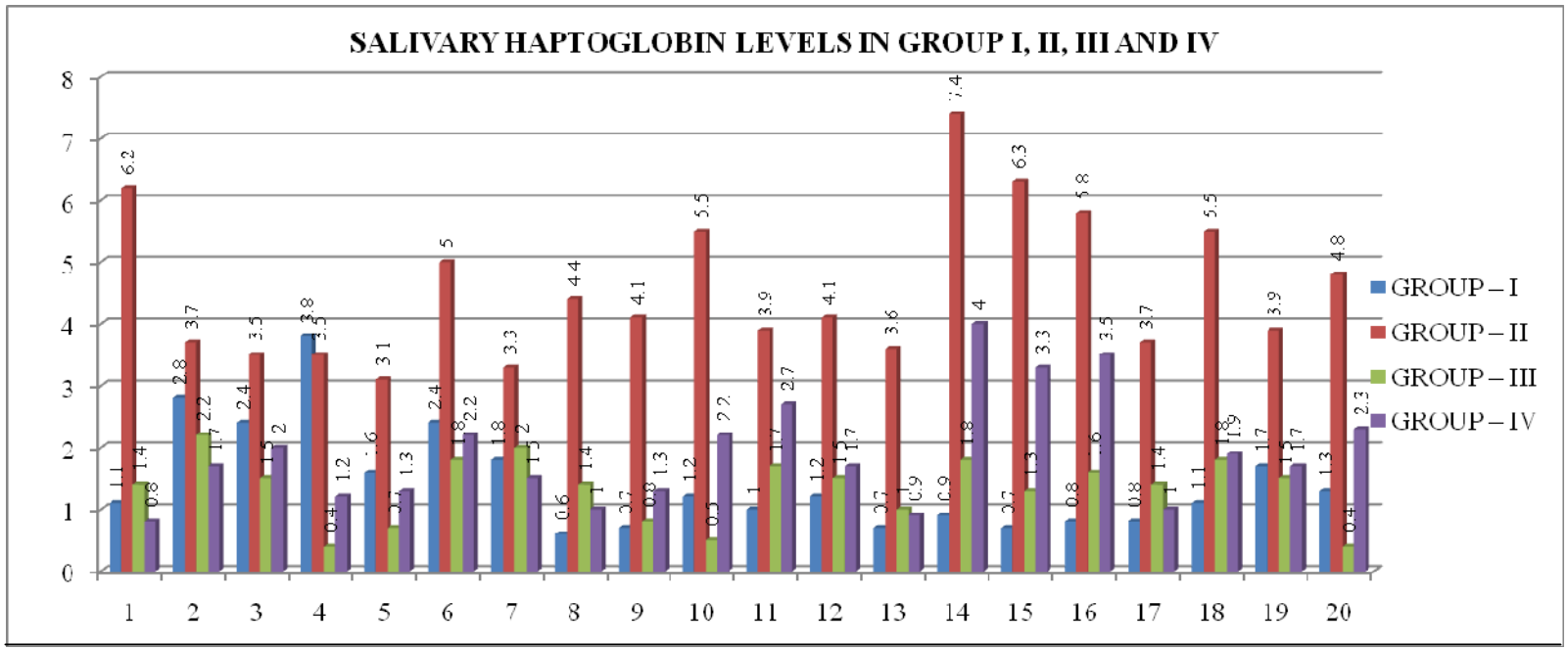
GRAPH – IX


SALIVARY IgG LEVELS IN GROUP I, II, III AND IV
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
GROUP – I
GROUP – II
GROUP – III
GROUP – IV

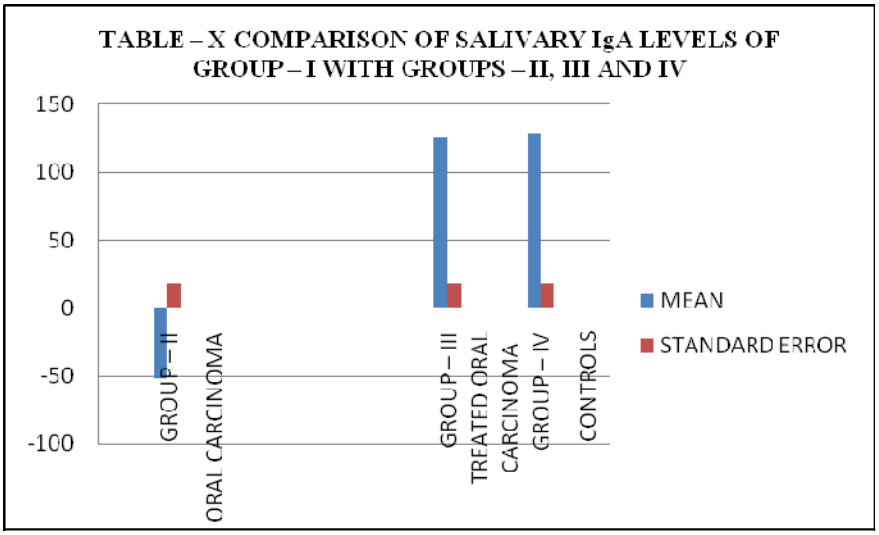
GRAPH – X


SALIVARY CRP LEVELS IN GROUP I, II, III AND IV
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
GROUP I
GROUP – II
GROUP – III
GROUP – IV

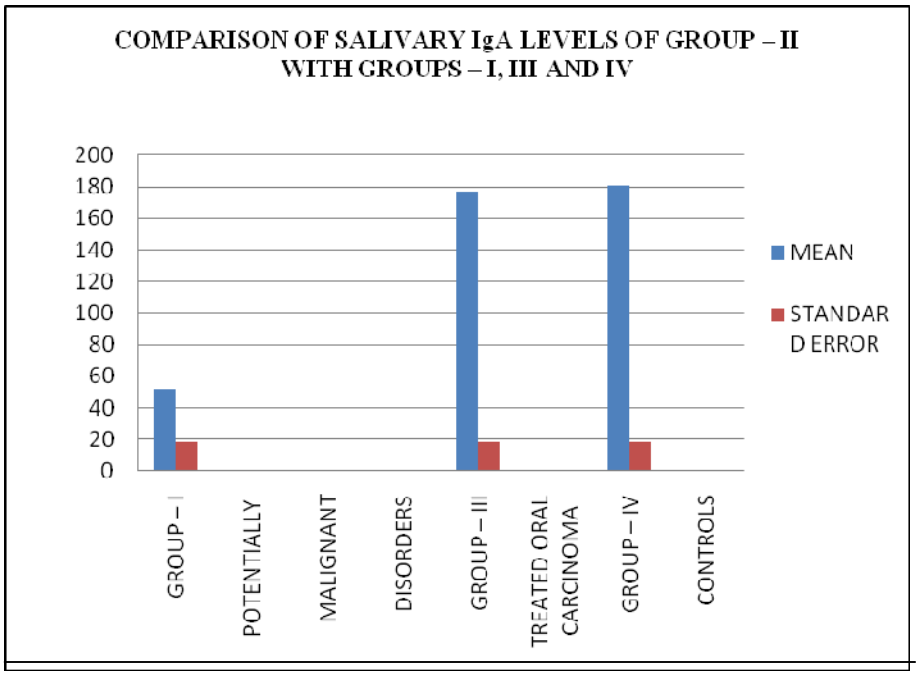
GRAPH – XI


SALIVARY HAPTOGLOBIN LEVELS IN GROUP I, II, III AND IV
GROUP – I
GROUP – II
GROUP – III
GROUP – IV

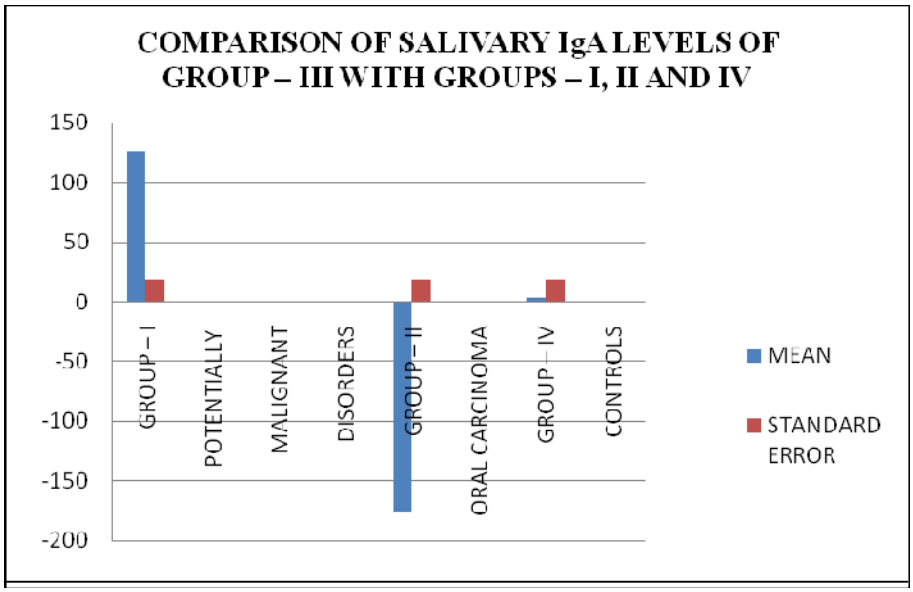
GRAPH – XII



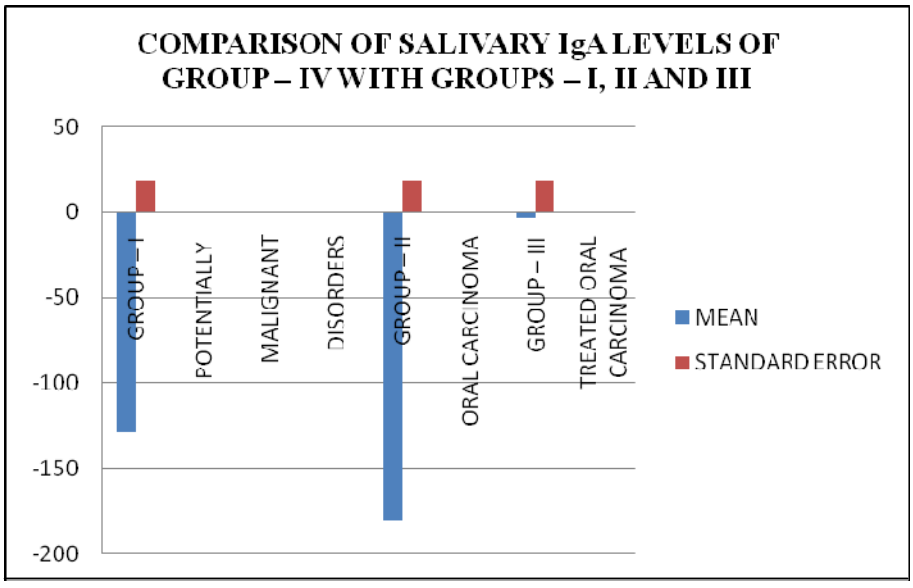
GRAPH – XIII



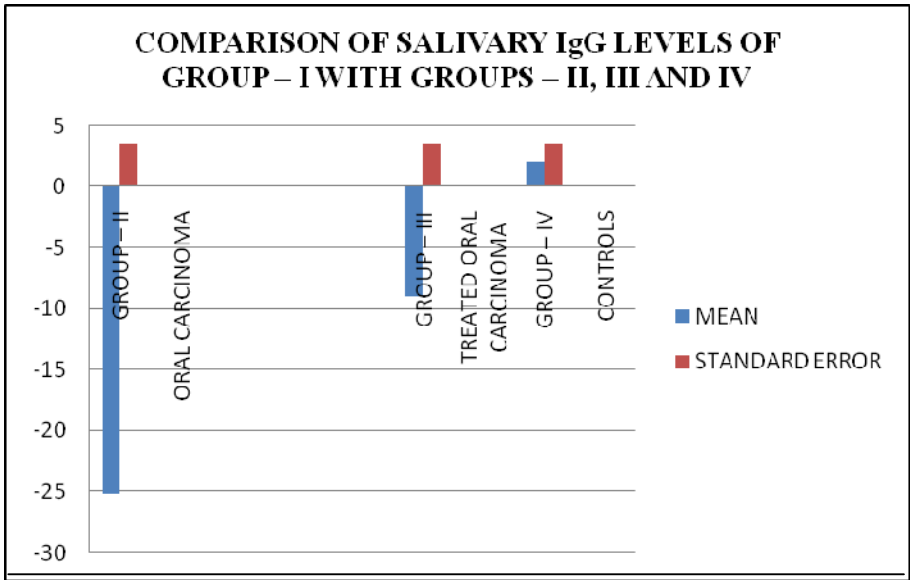
GRAPH – XIV



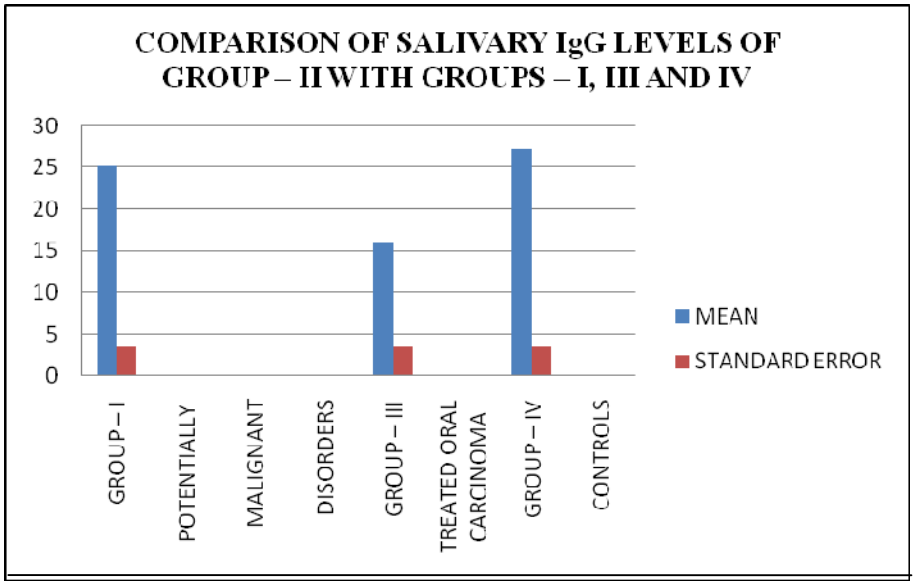
GRAPH – XV



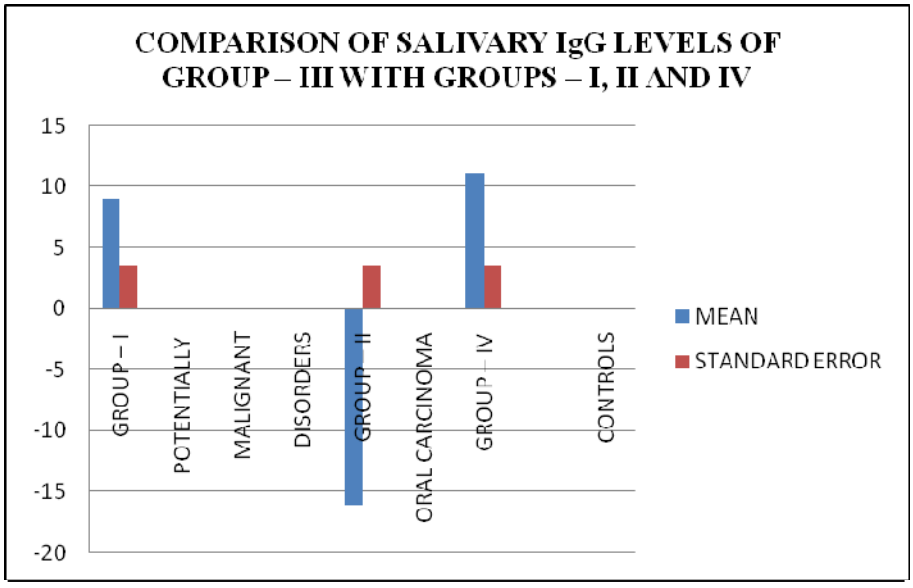
GRAPH – XVI



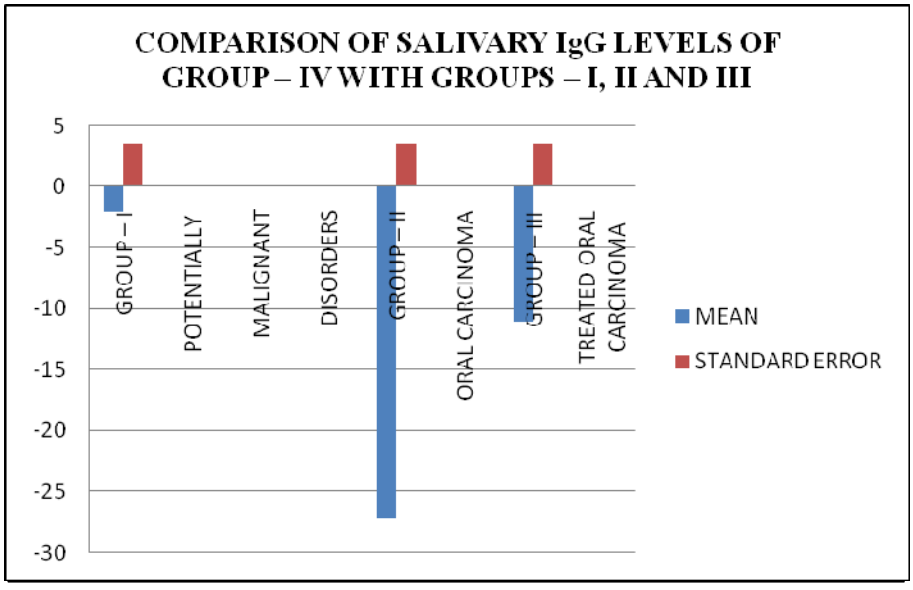
GRAPH – XVII



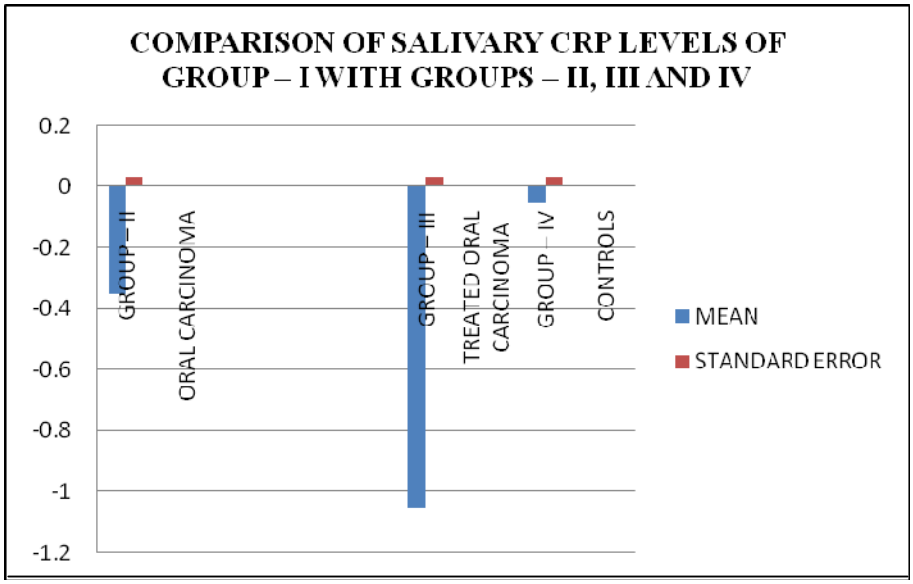
GRAPH – XVIII



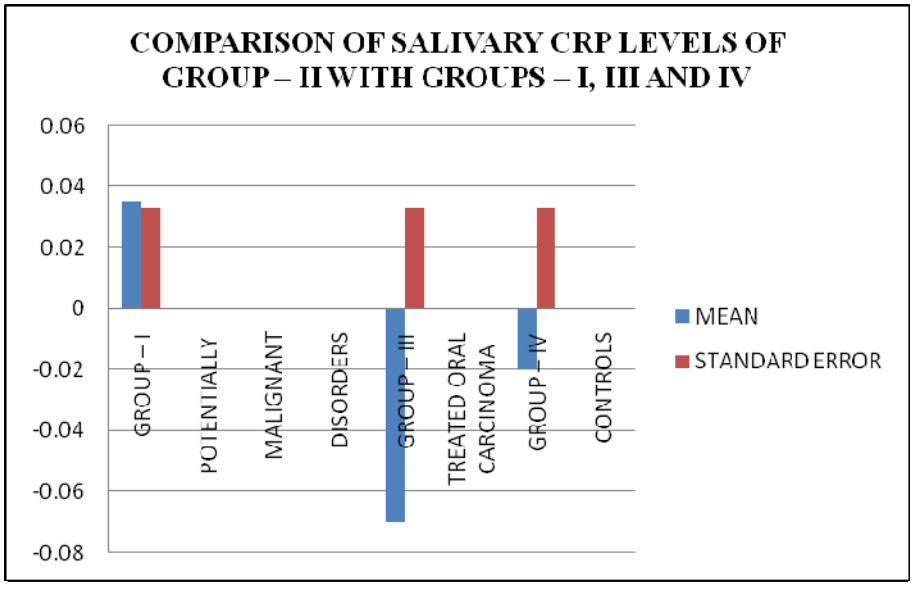
GRAPH – XVIX



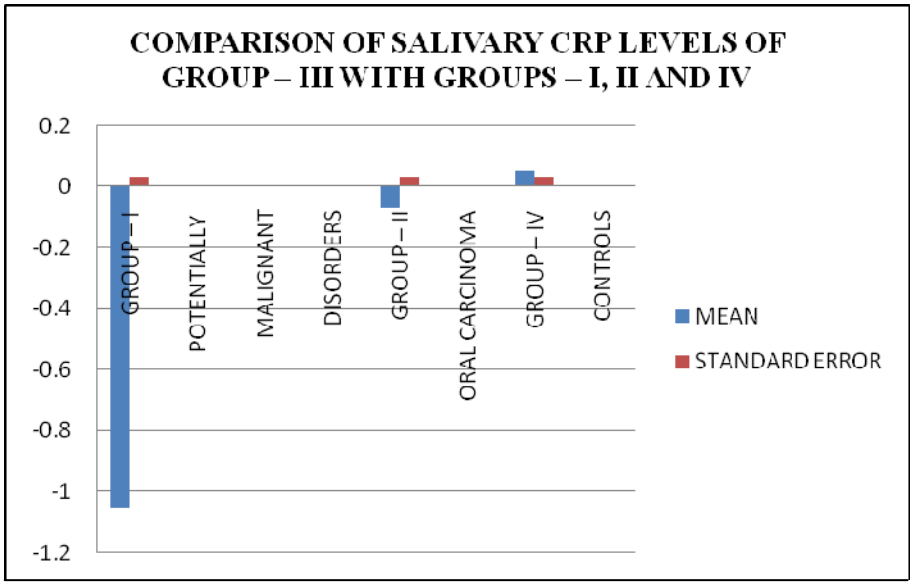
GRAPH – XX



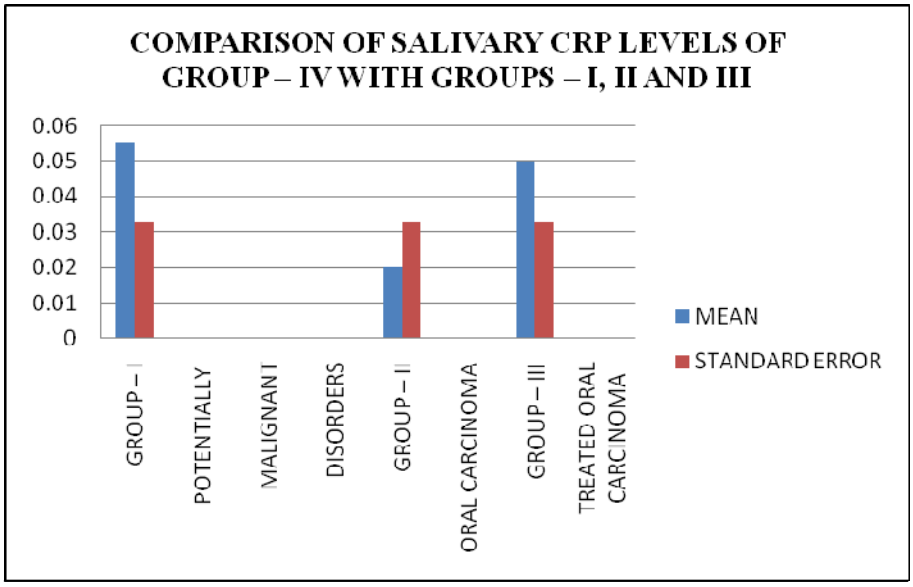
GRAPH – XXI



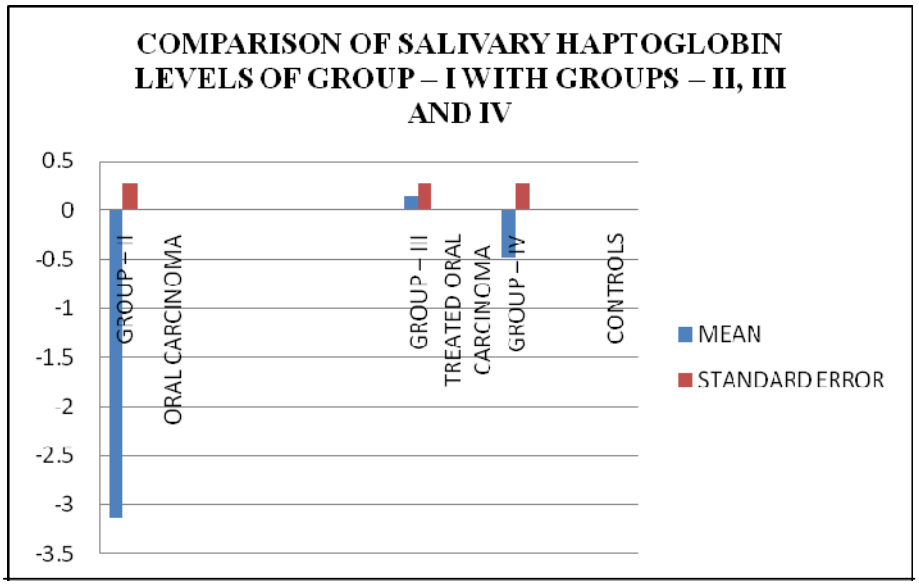
GRAPH – XXII



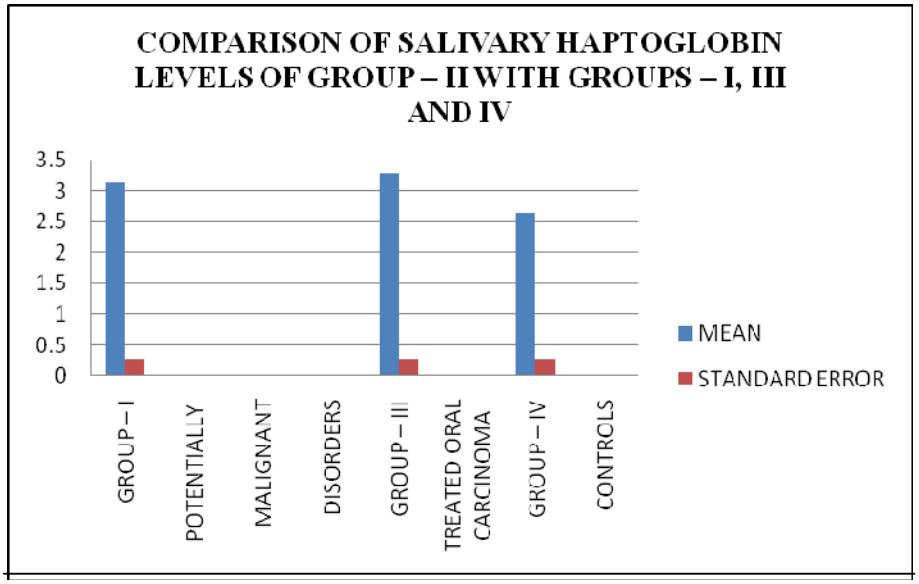
GRAPH – XXIII



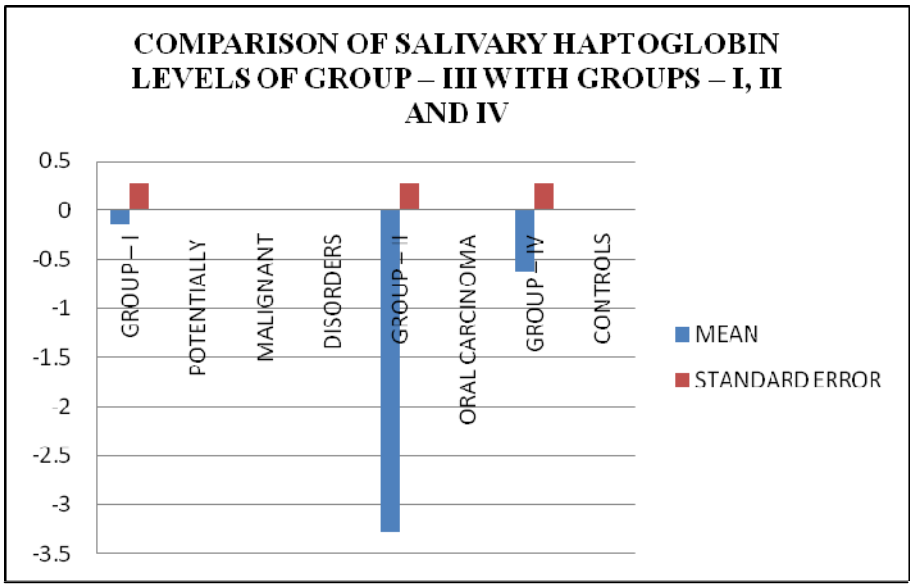
GRAPH – XXIV



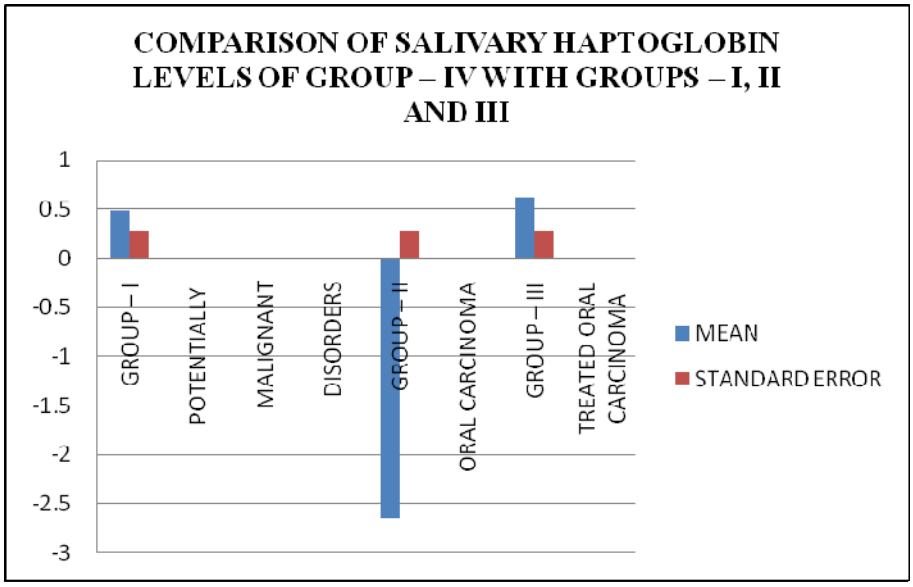
GRAPH – XXV



GRAPH – XXVI



GRAPH – XXVII



Oral cancer is one of the most deadly forms of cancer is the 6th commonest form of cancer worldwide. In India, it the most common type of cancer. Oral cancer is strongly associated with certain environmental and lifestyle risk factors, including tobacco, smoking, alcohol consumption, UV light and occupational exposures, and certain strains of viruses, such as the sexually transmitted human papilloma virus. These cancers are frequently aggressive in their biologic behaviour; patients with these types of cancer often develop a second primary tumour.[46]

The absence of definite early warning signs for most head and neck cancers suggests that sensitive and specific biomarkers are likely to be important in screening high-risk patients. Salivary analysis holds promise as a non-invasive approach to identify biomarkers for human oral cancer.[18, 36]

Salivary immunoglobulins play a role in host anti-tumor surveillance mechanisms. The predominant immunoglobulin in saliva is IgA. Approximately 90% of IgA in saliva is present as secretory IgA (S-IgA). Secretary immunoglobulin G (S-IgG) is present in saliva in very low quantities.[74] The Acute Phase Reactant Proteins (APRPs) are group of plasma proteins that alter their concentration in response to varied diseases. The levels of APRPs were shown to reflect events in tumour invasion and metastasis and were used in biochemical monitoring of cancer.[44]

This study deals with the salivary analysis to evaluate the immunological parameters like IgA, IgG, C-reactive protein and haptoglobin in the saliva of patients with potentially malignant disorders,

oral squamous cell carcinoma (OSCC) and patients treated for oral squamous cell carcinoma.

This study was conducted between April 2009 to May 2010 in the department of Oral Medicine and Radiology of Ragas Dental College and Hospital, Dr. Rai Memorial Medical and Cancer Centre, Chennai.

A case control study was conducted in which 80 subjects were selected. The study subjects were categorized into three groups: Group I consist of 20 patients who were suffering from potentially malignant disorders like Leukoplakia and Oral submucous fibrosis; Group II, 20 patients suffering from Oral cancer; Group III, 20 patients who were treated for oral cancer and Group IV, 20 normal controls.

Participants with infectious diseases during one month before saliva sampling, active dental abscesses', and collagen vascular diseases were excluded from the study.

Among the 80 subjects 62(77.5%) were males and 18(22.5%) were females. The minimum age of the study subjects was 19 years and the maximum age was 75 years.

In the present study, among the 80 subjects 20(25%) had the habit of smoking, 23(28.8%) had the habit of chewing, 12(15%) had the habit of chewing and smoking, 10(12.5%) had the habit of smoking and alcohol consumption,1(1.3%) had the habit of chewing and alcohol consumption and 3(3.8%) had all three habits together with a p-value of 0.000 which is statistically significant. Thus a positive correlation between smoking,

chewing, alcohol consumption and development of precancer and cancer has been established.

This is in accordance with various similar studies. Pindborg et al in 1984[59] performed a study on 100 subjects for their smoking and chewing habits and the condition of their oral mucosa. The predominant occurrence of oral submucous fibrosis was seen and the dominating habits were areca nut chewing and cigarette smoking and areca nut chewing and water pipe smoking in female population. Pindborg et al in 1997[60] examined 1866 individuals and found a positive correlation between leukoedema, preleukoplakia and leukoplakia and the habit of smoking. Salonen et al in 1990 [68] reported a positive correlation between tobacco use and leukoplakia on his study on 920 individuals. Sankaranarayanan in 1990[69] found a causal association between oral cancer and the chewing of betel quid containing tobacco leaves or stem and other tobacco habits. Rajendran.R et at in 1986[62] reviewed the etiology and pathogenesis of Oral submucous fibrosis. He suggested that certain customs or habits (chewing) prevalent among the population groups in South East Asia might be possible etiological factors. Shah N, Sharma PP in 1998[73] conducted a study to identify the role of chewing and smoking habit in the etiology of oral submucous fibrosis. In this study 236 cases of oral submucous fibrosis were compared with 221 control subjects matched for age, sex and socioeconomic conditions. It was found that chewing of areca nut, quid and pan masala was directly related to oral submucous fibrosis and not a single case was found without any

chewing habit. Crispian Scully et al in 2000[18] stated that the etiological factors of oral cancer include tobacco use, betel use (Bidi leaf, and often tobacco, plus spices, slaked lime, and areca nut) and alcohol consumption. Zain et al in 2001[92] stated about the role of tobacco smoking, chewing of tobacco, areca nut, and betel quid and drinking of alcohol are established cultural risk factors of oral pre-cancer and oral cancer worldwide. Saraswathi et al in 2006[70] stated that the habit of smoking, drinking and chewing tobacco products were common oral habits in India and these habits were positively related with development of oral lesion such as OSMF, leukoplakia and oral lichen planus which had potential for malignant transformation.

In the present study, the most common site for leukoplakia was in the retro-commissure area with 7(63.6%) subjects followed by buccal mucosa 2 (18.2%) and one each in the tongue and floor of the mouth. Jain et al in 1995[39] found that the most common site of leukoplakia was on the buccal mucosa 67.33% with p-value <0.01.

The present study showed all cases of OSMF in the buccal mucosa, with 5 (55.6%) in Grade III and 2 each in Grades I and IV and none in Grade II. Shah et al (1998)[73] stated that OSMF predominantly affects the buccal mucosa with the p-value <0.001.

In the present study among the total of 40 (100%) subjects, 11(27.5%) had carcinoma in the tongue, 17(42.5%) had in the buccal mucosa, 7(17.5%) had in the alveolar mucosa, 2(5%) had carcinoma in the

floor of the mouth and 1(2.5%) in the palate and 2(5%) had in both the tongue and the floor of the mouth. Oral cancer was mostly seen in the buccal mucosa, followed by tongue, alveolar mucosa, floor of the mouth and palate. Prabhu SR et al in 1988[61] found the most common site of oral cancer was buccal mucosa followed by tongue and other sites. Mehrota et al in 2003[51] stated that oral cancer was the commonest malignancy in Allahabad and buccal mucosa was the most common site of oral cancer.

Kayambe in 1999[43] performed a study in Congo and found palate to be the most frequent site and squamous cell carcinoma was most common type of cancer. This may be attributed to the different predominant habit of the subjects included in the study by Kayambe when compared to the subjects included in the present study and hence there is a different site predilection for oral carcinoma.

In the present study elevated levels of salivary IgA, IgG and Haptoglobin were observed in patients with potentially malignant disorders like leukoplakia and oral submucous fibrosis with a p-value of 0.000 which is significant. The mean salivary IgA in patients with potentially malignant disorders was 201.7 ± 76.01 µg/mL, the mean salivary IgG was 8.7± 4.07 µg/dL and the mean salivary haptoglobin was 1.43 ± 0.843 mg/dL. In controls the mean salivary IgA was 72.87 ± 26.46 µg/mL, the mean salivary IgG 6.58 ± 5.60 µg/dL and the mean salivary haptoglobin was 1.91 ± 0.895 mg/dL. This is in accordance with the similar studies conducted by Phatak AG and Gosavi DK 1975[58] (p-value of <0.001) Thomas Loning 1979[81],

Rajendran 1986[62] (p-value <0.001) and Krasteva 2008[46] (IgA: p-value of 0.043 and IgG: p-value of 0.018). Phatak AG and Gosavi DK in 1975[58] reported that the total IgG was significantly elevated in OSMF patients 2009 ± 258mg/100mL when compared to that in controls 1708 ± 346mg/100mL with a p-value of <0.001. Thomas Loning et al in 1979[81] found that the incidence of immunoglobulins (IgA and IgG) was twice as high in those cases of leukoplakia where dysplasia was present but no numerical values were given. Rajendran et al in 1986[62] found a significant rise in IgA levels with p-value of <0.001 in patients with oral submucous fibrosis. Krasteva.A et al in 2008[46] found that salivary IgA and IgG were significantly increased in patients with precancerous lesions. The salivary IgA levels were 168mg/L when compared to the controls 83mg/L with p-value of 0.043 and salivary IgG levels were 70mg/L in precancerous lesions, in controls 22mg/L with p-value of 0.018.

The present study showed that there was a significant rise in the salivary IgA, IgG, and Haptoglobin with a p-value of 0.000 and a slight elevation in the salivary CRP levels in patients with oral carcinoma with a p-value of 0.021 which is significant. The mean salivary IgA in patients with oral carcinoma was 253.35 ± 83.3µg/mL, the mean salivary IgG was 33.86 ± 20.31mg/dL, mean salivary CRP was 0.050 ± 0.10 µg/mL and the mean salivary haptoglobin was 4.56 ± 1.19mg/dL. In controls the mean salivary IgA was 72.87 ± 26.46 µg/mL, the mean salivary IgG 6.58 ± 5.60 µg/dL, the mean salivary CRP was 0.070 ± 0.13 mg/L and the mean salivary

haptoglobin was 1.91 ± 0.895 mg/dL. This is in accordance with similar studies by Mark.A 1973[49] (mean IgA: 2.15± 0.65mg with p-value <0.001), Ashley M. Brown 1975[04] (p-value-0.01), Rajendran 1986[62] (p-value<0.0001), Hu-De-En 1987[38] (p-value<0.01), Gallo.O 1994[30] (IL-6 and CRP r = 0.69 with p-value of 0.0001), Dritan Turhani 2005[20], Kashmoola 2001[42]and Al-Rawi 2005[03], Krasteva 2008[46] (IgA and IgG: p-value 0.000, CRP: p-value 0.004 and haptoglobin: p-value 0,006) and Sunil.D.Khandavilli 2009[80] (p = 0.003). Mark.A et al in 1973[49] found that the highest IgA titers were seen in patients with oral and pulmonary cancers with the mean IgA: 2.15± 0.65mg which was 3.5 times that of the control group with a p-value <0.001. Ashley M. Brown et al in 1975[04] stated that the IgA content of whole saliva of cancer patients was significantly elevated above that of controls. The elevation of saliva when compared to control patients was significant at the .01 level. Rajendran et al in 1986[62] found a significant rise in IgA levels with p-value of <0.0001 in oral cancer patients. He also stated that the immunological derangements were more pronounced in oral cancer than in OSMF. Hu – De – En et al in 1987[38] found that there was a tendency for higher levels of immunoglobulins to be associated with more advanced stage of carcinoma. The IgA levels were 295 ± 112mg% and IgG levels were 2076 ± 587mg% with p-value of <0.01. Gallo.O et al in 1994[30] conducted a study in 18 patients with oral squamous cell carcinoma and found an increase in IL-6 which in turn induces the synthesis of CRP in head and neck carcinoma. Significant (P < 0.0001) relationships were found

between IL-6 and CRP (r = 0.69). Dritan Turhani et al in 2005[20] has demonstrated an increase in the levels of CRP in oral squamous cell carcinoma patients. Kashmoola et al in 2001[42] and Al-Rawi et al in 2005[03] had showed that patients with oral squamous cell carcinoma had markedly increased salivary total protein concentration which may be due to increasing salivary immunoglobulins. Numerical values were not given for discussion. Krasteva.A et al in 2008[46] found that the salivary levels of IgA, IgG, CRP and Haptoglobin in patients with oral carcinoma were significantly increased. Salivary IgA levels were 152mg/L with p-value 0.000, Salivary IgG levels were 38mg/L p-value 0.000, CRP levels were 0.157mg/L p-value 0.004 and Haptoglobin levels were 30 with p-value 0.006. Sunil.D. Khandavilli et al in 2009[80] did a study which was designed to establish if elevated preoperative levels of CRP could predict the prognosis of patients treated with primary surgery for oral squamous cell carcinoma (SCC). He stated that a raised preoperative CRP with p-value of 0.003 was associated with worse overall survival.

The studies by Thomas Loning 1979[81], Robino Muchado de Souza 2003[67] (salivary IgA : 13.7± 3.9 mg/dL) and Shpitzer.T 2007[74] (salivary IgG 125%, P = 0.01, while S-IgA was lower by 45% P = 0.001) are not in accordance with the present study. Thomas Loning et al in 1979[81] found that the concentration of IgA and IgG decreased significantly with tumor dedifferentiation. Robino Muchado de Souza et al in 2003[67] found that salivary IgA levels were reduced in cancer patients and were related to

malnutrition, stress and tobacco. He found that the mean salivary IgA level was 17.0 ± 10.4mg/dL in controls and 13.7± 3.9 mg/dL in oral cancer patients. Shpitzer.T et al in 2007[74] utilized comprehensive salivary analysis to evaluate biochemical and immunological parameters in the saliva of oral squamous cell carcinoma patients. He found that the concentration of salivary IgG was higher by 125% in oral squamous cell carcinoma patients while the concentration of secretory IgA was lowered by 45%. This may be attributed to the small sample size in the present study, the age of the patients and the immune status of the patients.

In the present study the salivary IgA, IgG and CRP in treated oral cancer patients remained significantly higher with a p-value of 0.000 which is significant. The mean salivary IgA in patients with treated oral carcinoma was 76.26 ± 9.20 µg/mL, the mean salivary IgG was 17.74 ± 3.12 µg/dL and the mean salivary CRP was 0.120 ± 0.128mg/L. In controls the mean salivary IgA was 72.87 ± 26.46 µg/mL, the mean salivary IgG 6.58 ± 5.60 µg/dL and the mean salivary CRP was 0.070 ± 0.13 mg/L. This is in accordance with similar studies by Einhorn 1972[22](p-value <0.01) Brown 1981[11] ($p<0.03$) Jankovic 1995[40] (mean salivary IgG :0.095 g/l) and Krasteva.A 2008[46] ($p<0.05$). Einhorn et al in 1972[22] observed elevated levels of IgG at the time of completion of radiotherapy and higher levels 3 months after radiotherapy. This may represent enhanced immunization by antigens released during radiation induced tumour breakdown. Brown et al in 1981[11] found that following radiotherapy changes in specific

agglutination titers of oral isolates reflected changes in saliva IgA. He found 13 patients with increased salivary IgA with p-value of <0.03 when he assessed 36 patients over a 30 month post-irradiation period. Jankovic.L et al in 1995[40] in his study on 40 patients with neoplastic disorders who have been treated has found that the mean IgG/IgA salivary ratio was 1.27 (normally below 1.0) due to an increased salivary concentration of IgG (mean 0.095 g/l). Krasteva.A et al in 2008[46] found that the salivary levels of IgG and IgA remain significantly higher ($p<0.05$) in treated cancer patients.

The studies conducted by Thomas Loning 1979[81], Hu-De-En 1987[38] (p-value of <0.01) and Meurman JH 1997[53] (p-value<0.001) were not in accordance with the present study. Thomas Loning et al in 1979[81] found that the immunoglobulins (IgA and IgG) levels decreased after radiation therapy. Hu De-En et al in 1987[38] reported that the levels of IgG decreased markedly after radiation therapy. IgG levels were 2076 ± 567 mg% in oral cancer patients and in patients treated with radiotherapy the IgG levels decreased 1901 ± 688mg% with a p-value of <0.01. Meurman JH et al in 1997[53] found that the total salivary IgA decreased during cancer therapy which returned to the baseline level after termination of the treatment. The mean IgA was 70.5 +/- 52.8 mg/mL at baseline, 35.8 +/- 15.0 mg/mL after radiotherapy with p – value less than 0.001. This may be due to various factors such as the age of the patients, the immune status of the patients and the duration between the completion of the treatment and the sample collection.

The present study showed a significant decrease in the salivary haptoglobin levels in treated oral cancer patients. The mean salivary haptoglobin in treated oral cancer patients was the lowest 1.285 ± 0.492 mg/dL and the mean salivary haptoglobin was highest in patients with oral carcinoma 4.56 ± 1.19mg/dL with a p-value of 0.000. This is in accordance with the study conducted by Krasteva 2008[46] (p-value0.007). Krasteva.A et al in 2008[46] found that there was a significant decrease in the salivary haptoglobin in treated oral cancer patients when compared with the oral cancer patients with a p-value of 0.007. There is no sufficient data in the literature to emphasise the cause for the decrease in haptoglobin in patients treated for oral carcinoma.

The present study titled "Estimation of salivary immunoglobulins and acute phase proteins in patients with Potentially malignant disorders, Oral Cancer and Treated oral cancer" was conducted in the department of Oral Medicine and Radiology, Ragas Dental College, Uthandi, Chennai and Dr. Rai Memorial Medical and Cancer Centre, Chennai, to estimate the salivary levels of IgA, IgG, C-reactive protein and Haptoglobin in patients with potentially malignant disorders like leukoplakia and oral submucous fibrosis, oral cancer and treated oral cancer and to compare the values with the control subjects.

A total of 80 individuals were selected for the study. Among the study subjects 20 patients were suffering from Potentially Malignant Disorders, 20 patients were suffering from Oral Cancer, 20 were treated for Oral Carcinoma and 20 patients were normal controls. Informed consent was taken from all subjects before including them in the study. Participants with infectious diseases during one month before saliva sampling, active dental abcesses, and collagen vascular diseases were excluded from the study.

The experimental subjects were made to sit comfortably on a Dental Chair. Sterile hand gloves were used during examination of the patients. Patients were examined under halogen lamp in the dental chair under aseptic conditions and relevant demographic data were collected. Clinical diagnosis was made and patients who showed characteristic features of Leukoplakia, Oral submucous fibrosis and Oral Cancer were prepared for sample collection.

The patients were asked to rinse their mouth with water and were made to sit comfortably in a dental chair. Saliva was collected during a 15-minutes interval by spitting method. This was pooled saliva and represented the output from all the salivary glands. 2-3mL of saliva was collected in sterile containers. All samples were kept in ice after collection and then centrifuged at 3000 rpm for 10 min to remove particulate materials and the supernatant was used for estimation of the immunoglobulins and acute phase proteins.

The study documents the following data:

- Among the 80 subjects 62(77.5%) were males and 18(22.5%) were females. The minimum age of the study subjects was 19 years and the maximum age was 75 years.
- Among the 80 subjects 20(25%) had the habit of smoking, 23(28.8%) had the habit of chewing, 12(15%) had the habit of chewing and smoking, 10(12.5%) had the habit of smoking and alcohol consumption, 1(1.3%) had the habit of chewing and alcohol consumption and 3(3.8%) had all the three habits together with a p-value of 0.000 which is statistically significant. Thus a positive correlation between smoking, chewing, alcohol consumption and development of precancer and cancer has been established.

- The most common site for leukoplakia was in the retro-commissure area with 7 (63.6%) subjects followed by buccal mucosa 2(18.2%) and one each in the tongue and floor of the mouth.
- The present study showed all 9 cases of OSMF in the buccal mucosa with 5 (55.6%) in Grade III and 2 each in Grades I and IV and none in Grade II.
- Among the total of 40 (100%) subjects in Groups II and III, 11(27.5%) had carcinoma in the tongue, 17(42.5%) had in the buccal mucosa, 7(17.5%) had in the alveolar mucosa, 2(5%) had carcinoma in the floor of the mouth and 1(2.5%) in the palate and 2(5%) had in both the tongue and the floor of the mouth.
- Elevated levels of salivary IgA, IgG, CRP and Haptoglobin were observed in patients with potentially malignant disorders like leukoplakia and oral submucous fibrosis. The mean salivary IgA in patients with potentially malignant disorders was 201.7 ± 76.01 µg/mL, the mean salivary IgG was 8.7± 4.07 µg/dL, mean salivary CRP was 0.015± 0.036 µg/mL and the mean salivary haptoglobin was 1.43 ± 0.843 mg/dL. In controls the mean salivary IgA was 72.87 ± 26.46 µg/mL, the mean salivary IgG 6.58 ± 5.60 µg/dL, the mean salivary CRP was 0.070 ± 0.13 mg/L and the mean salivary haptoglobin was 1.91 ± 0.895 mg/dL.
- There was a significant rise in the salivary IgA, IgG, and Haptoglobin and a slight elevation in the salivary CRP levels in patients

with oral carcinoma. The mean salivary IgA in patients with oral carcinoma was 253.35 ± 83.3µg/mL, the mean salivary IgG was 33.86 ± 20.31mg/dL, mean salivary CRP was 0.050 ± 0.10 µg/mL and the mean salivary haptoglobin was 4.56 ± 1.19mg/dL. In controls the mean salivary IgA was 72.87 ± 26.46 µg/mL, the mean salivary IgG 6.58 ± 5.60 µg/dL, the mean salivary CRP was 0.070 ± 0.13 mg/L and the mean salivary haptoglobin was 1.91 ± 0.895 mg/dL.

- The salivary IgA, IgG and CRP in treated oral cancer patients remained significantly higher. The mean salivary IgA in patients with treated oral carcinoma was 76.26 ± 9.20 µg/mL, the mean salivary IgG was 17.74 ± 3.12 µg/dL and the mean salivary CRP was 0.120 ± 0.128mg/L. In controls the mean salivary IgA was 72.87 ± 26.46 µg/mL, the mean salivary IgG 6.58 ± 5.60 µg/dL and the mean salivary CRP was 0.070 ± 0.13 mg/L.
- A significant decrease in the salivary haptoglobin levels were observed in treated oral cancer patients. The mean salivary haptoglobin in treated oral cancer patients was 1.285 ± 0.492 mg/dL and the mean salivary haptoglobin in controls was 1.91 ± 0.895 mg/dL.

Thus the salivary IgA was found to be highest in patients with oral carcinoma followed by patients with potentially malignant disorders, then treated oral cancer patients and the controls having the lowest values. The salivary IgG was found to be highest in patients with oral carcinoma followed by patients treated for oral carcinoma, patients

with potentially malignant disorders and lowest in the controls. The salivary CRP was highest in the patients treated for oral carcinoma followed by controls, patients with oral carcinoma and lowest in patients with potentially malignant disorders. The salivary haptoglobin was highest in oral carcinoma patients followed by controls, patients with potentially malignant disorders and lowest in patients treated for oral carcinoma.

The increase of these salivary immunoglobulins and acute phase proteins in precancerous lesions and oral carcinoma may reflect the local inflammation accompanying the neoplastic process in the oral cavity or due to the direct transudation of these proteins from the blood or may be considered as a local defense mechanism against tumour development.

To conclude, a statistically significant difference was observed in relation to the tested parameters between the four different groups included in the study. Salivary analysis holds promise as a non-invasive approach to identify biomarkers for human oral cancer. An extensive, well-executed study is required to ensure the practical usefulness of these biomarkers in screening for early oral cancer, possible recurrent disease and individuals with high risk of oral malignancy. Further studies should include a larger sample size to emphasize the sensitivity and specificity of these biomarkers so as to arrive at an early diagnosis aimed at the betterment of the patients.

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MASTER CHART

GROUP I – POTENTIALLY MALIGNANT LESIONS

S.NO	NAME	AGE	SEX	HABIT	LESION	SALIVARY IgA	SALIVARY IgG	SALIVARY CRP	SALIVARY HAPTOGLOBIN
1	Madhan kumar	27	Male	Chewing	OSMF	132.92	7.6	0.1	1.1
2	Sagayam	48	Male	Smoking	Leukoplakia	156.24	1.3	0	2.8
3	Arumugam	43	Male	Smoking Chewing	Leukoplakia	287.8	14.7	0	2.4
4	Pushpalingam	40	Male	Smoking Alcohol	Leukoplakia	317.10	13.0	0	3.8
5	Mymoon Beevi	36	Female	Chewing	OSMF	113.76	5.5	0	1.6
6	Parthiban	21	Male	Smoking Chewing	Leukoplakia	150.84	15.8	0	2.4
7	Sangram	24	Male	Chewing	OSMF	142.63	15.0	0	1.8
8	Vijaya	30	Female	Chewing	OSMF	243.69	5.3	0	0.6
9	Ganesh	38	Male	Smoking Alcohol	Leukoplakia	133.9	6.9	0	0.7
10	Venkatesh	24	Male	Chewing	OSMF	161.55	15.3	0	1.2
11	Munuswamy	72	Male	Smoking Alcohol	Leukoplakia	180.6	9.6	0	1.0
12	Gopu	75	Male	Smoking	Leukoplakia	113.17	6.4	0	1.2
13	Arun	27	Male	Smoking Chewing	Leukoplakia	230.74	5.5	0	0.7
14	Vinayagam	65	Male	Smoking	Leukoplakia	237.13	5.3	0	0.9
15	Prakash	24	Male	Chewing	OSMF	241.6	9.2	0	0.7
16	Thanikachalam	24	Male	Chewing	OSMF	108.79	9.2	0	0.8
17	Selvam	33	Male	Chewing Alcohol	OSMF	161	5.9	0	0.8
18	Basha	40	Male	Chewing	OSMF	296.6	8.1	0.1	1.1
19	Muralikumar	24	Male	Smoking Chewing	Leukoplakia	361.37	8.9	0	1.7
20	Tamilmani	69	Male	Smoking	Leukoplakia	262.8	5.5	0.1	1.3

GROUP II – ORAL CARCINOMA

S.NO	NAME	AGE	SEX	HABIT	CARCINOMA SITE	SALIVARY IgA	SALIVARY IgG	SALIVARY CRP	SALIVARY HAPTOGLOBIN
1	Vatchala	42	Female	Chewing	Alveolar mucosa & buccal sulcus	141.44	22	0.1	6.2
2	Sridharan	44	Male	Smoking	Tongue	125.71	15.2	0	3.7
3	Ramakrishnan	42	Male	Smoking Chewing	Tongue	338.98	20	0	3.5
4	Subramaniyan	61	Male	Smoking	Buccal mucosa	380.34	11	0	3.5
5	Arogyadass	47	Male	Chewing	Alveolar mucosa & buccal sulcus	273.73	29	0.2	3.1
6	Radhakrishnan	56	Male	Smoking Chewing Alcohol	Buccal mucosa	124.42	55.8	0.1	5.0
7	Selvaraj	63	Male	Smoking Alcohol	Tongue & Floor of the mouth	139.23	12.0	0	3.3
8	Guruswamy	35	Male	Smoking	Buccal mucosa	268.23	14.2	0	4.4
9	Selvaraj	40	Male	Smoking Alcohol	Buccal mucosa	246.09	23.3	0	4.1
10	Godavari	53	Female	Chewing	Alveolar mucosa & buccal sulcus	330.23	18.8	0	5.5
11	Vetrivel	60	Male	Smoking Chewing	Buccal mucosa	208.66	4.1	0	3.9
12	Madurai Muthu	55	Male	Smoking Chewing	Tongue	237.33	53.4	0	4.1
13	Yesammal	50	Female	Chewing	Buccal mucosa	250.42	57.0	0	3.6
14	Kumarappan	33	Male	Smoking	Tongue	273.08	68.3	0	7.4
15	Kabir	37	Male	Smoking Alcohol	Palate	356.07	55.0	0.4	6.3
16	Padmavathy	40	Female	Chewing	Buccal mucosa	365.78	48.6	0.1	5.8

17	Varadharajan	45	Male	Smoking Chewing Alcohol	Floor of the mouth	295.74	52.3	0	3.7
18	Jawahar	60	Male	Chewing	Floor of the mouth	153.45	66	0	5.5
19	Mary	38	Female	Chewing	Buccal mucosa	234.46	14.5	0.1	3.9
20	Chandrasekaran	67	Male	Smoking	Tongue	324.64	25.7	0	4.8

GROUP III – TREATED ORAL CARCINOMA

S.NO	NAME	AGE	SEX	HABIT	CARCINOMA SITE	SALIVARY IgA	SALIVARY IgG	SALIVARY CRP	SALIVARY HAPTOGLOBIN
1	Ramanathan	62	Male	Smoking	Tongue	67.45	25.4	0	1.4
2	Sethuraman	69	Male	Smoking	Buccal mucosa	82.0	16.7	0	2.2
3	Vaidyanathan	24	Male	Smoking Alcohol	Alveolar mucosa & buccal sulcus	62.3	15.4	0.1	1.5
4	Renuka	34	Female	Chewing	Buccal mucosa	73.4	18.5	0.1	0.4
5	Logu	49	Male	Smoking Chewing Alcohol	Tongue	66.0	16	0.4	0.7
6	Abhraham	42	Male	Smoking	Buccal mucosa	72.65	25.0	0.2	1.8
7	Murugan	39	Male	Smoking Alcohol	Tongue	79.78	16.5	0.1	2
8	Venkatesh	30	Male	Smoking	Tongue	64.5	17.5	0	1.4
9	Devendran	55	Male	Smoking	Buccal mucosa	79.8	14.6	0	0.8
10	Pachaiammal	57	Female	Chewing	Alveolar mucosa & buccal sulcus	68.4	13.8	0.2	0.5
11	Madhan	31	Male	Smoking Chewing	Buccal mucosa	78.4	17.4	0.1	1.7
12	Kadeeresan	70	Male	Smoking Alcohol	Buccal mucosa	89.0	17.57	0.1	1.5
13	Soorya Narayanan	36	Male	Smoking Chewing	Tongue	98.3	18.4	0.4	1.0
14	Manjula	42	Female	Chewing	Buccal mucosa	83.7	21.30	0.2	1.8
15	Mukesh kumar	40	Male	Smoking	Buccal mucosa	79.2	20.2	0.1	1.3
16	Duraivel	65	Male	Chewing	Tongue & Floor of the mouth	77.8	15.0	0	1.6
17	Mary Fernandes	53	Female	Chewing	Tongue	75.28	17.6	0	1.4

18	Pandiyan	40	Male	Smoking Chewing	Alveolar mucosa & buccal sulcus	87.8	15.0	0.1	1.8
19	Devi	60	Female	Chewing	Alveolar mucosa & buccal sulcus	72.8	16.3	0.3	1.5
20	Sakunthala	43	Female	Chewing	Buccal mucosa	67	16.7	0	0.4

GROUP IV – CONTROL GROUP

S.NO	NAME	AGE	SEX	HABIT	SALIVARY IgA	SALIVARY IgG	SALIVARY CRP	SALIVARY HAPTOGLOBIN
1	Vinodh	28	Male	Smoking	88.21	27.9	0.5	0.8
2	Kamarunisha	25	Female	-	107	4.8	0	1.7
3	Vedachalem	60	Male	-	109.2	6.0	0.1	2.0
4	Krishnan	53	Male	-	47.6	8.2	0.1	1.2
5	Yuga	19	Male	Chewing	43.7	5.5	0	1.3
6	Issac	41	Male	Smoking Alcohol	33.3	5.4	0.1	2.2
7	Sundareshwaran	36	Male	-	79	6.7	0	1.5
8	Prakash	40	Male	Smoking	40.3	5.6	0	1.0
9	Rajasekaran	68	Male	-	32.6	6.5	0	1.3
10	Juliya	26	Female	-	43.3	3.4	0	2.2
11	Guru	35	Male	Smoking Chewing	40.6	3.7	0	2.7
12	Selvam	33	Male	Smoking	83.6	1.5	0.3	1.7
13	Lakshmi	50	Female	-	103	3.8	0	0.9
14	Muthukumar	39	Male	-	82.7	5.5	0.2	4
15	Gopu	23	Male	-	74.84	10.3	0	3.3
16	Rajendran	65	Male	-	103.85	2.8	0	3.5
17	Devaraj	58	Male	Smoking	87.78	7.2	0	1.0
18	Preetham	40	Male	Smoking	81.67	1.3	0	1.9
19	Saraswathi	30	Female	Chewing	81.2	3.0	0.1	1.7
20	Baby	44	Female	-	94.24	13.5	0	2.3

PROFORMA

RAGAS DENTAL COLLEGE & HOSPITAL
2/102, EAST COAST ROAD, UTHANDI, CHENNAI – 600 119.
DEPARTMENT OF ORAL MEDICINE & RADIOLOGY

Estimation of salivary immunoglobulin and acute phase proteins in patients with potentially malignant disorders, untreated and treated oral cancer.

Serial no. Op. no.

Name: Age/ Sex:

Religion:

Occupation: Income:

Address: Phone no**:**

HABITS	**PRESENT**	**ABSENT**
Smoking		
Chewing		
Alcohol		

LESION	PRESENT	ABSENT
Potentially malignant disorders		
Untreated Oral Squamous Cell Carcinoma		
Treated Oral Squamous Cell carcinoma		

Date of sample collection:

PARAMETERS/ LESION	SALIVARY IgA (mg/l)	SALIVARY IgG (mg/l)	SALIVARY CRP (mg/l)	SALIVARY HAPTOGLOBIN (mg/l)
Potentially malignant disorders				
Untreated Oral Squamous Cell Carcinoma				
Treated Oral Squamous Cell carcinoma				

CONSENT FORM

CONSENT LETTER

I _____ the undersigned hereby give my consent for the performance of diagnostic test on myself "to evaluate the prognostic value of salivary immunoglobulin and acute phase proteins in potentially malignant disorders and in oral cancer" conducted by Dr. S. Aswini under the able guidance of Dr. S. Shanmugam M.D.S., Professor and HOD, Department of Oral Medicine and Radiology, Ragas Dental College and Hospital, Chennai. I have been informed and explained the status of my disorder, evaluation procedure, risk involved and likelihood of success. I also understand and accept this as a part of study protocol, thereby voluntarily, unconditionally, freely give my consent without any fear or pressure in mentally sound and conscious state to participate in the study.

Witness/ Representative
(If any)

Patient signature

Date:

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«ñ½ñ¢ âùè¢° âù¢Â¬ìò «ï£ò¤ù¢ îù¢¬ñ¬òð¢ ðø¢ø¤»ñ¢, Þð¢ðó¤«ê£î¬ù ðø¢ø¤»ñ¢, Üîù£ô¢ ãø¢ðìè¢Ã®ò õ¤¬÷¾è¬÷ð¢ ðø¢ø¤»ñ¢ â´î¢¶ Ãøð¢ðì¢´÷¢÷¶ âù¾ñ¢, Þï¢î ðó¤«ê£î¬ùè¢°, ï£ù¢ âï¢îõ¤î Üê¢êºñ¤ù¢ø¤ îù¢ù¤ê¢¬êò£è¾ñ¢, ªî÷¤õ£ù ºî¶ùù¢ âù¢Â¬ìò ðó¤Ìóí êñ¢ñî¤î¢î¬ù Ü÷¤è¢è¤«øù¢ âù Þîù¢ Íôñ¢ ªîó¤òð¢ð´î¢¶è¤«øù¢.

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